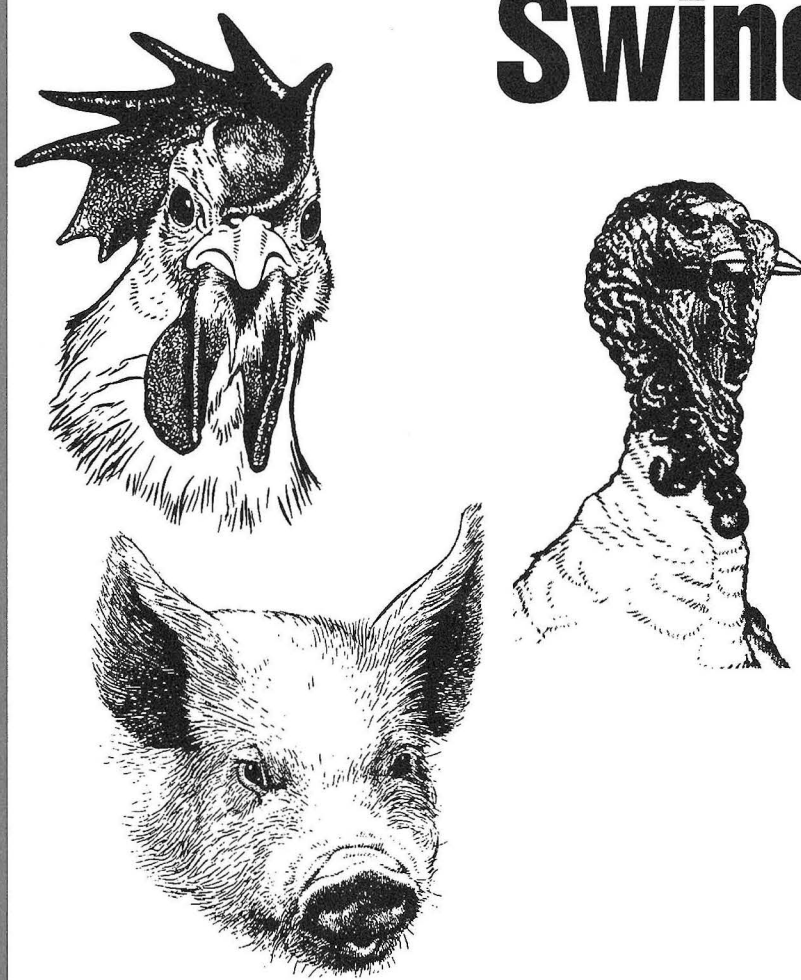
Department of Animal Sciences

Research and Reviews: Poultry and Swine



THE OHIO STATE UNIVERSITY OARDC

THE OHIO STATE UNIVERSITY EXTENSION

December 1999
Special Circular 171
Ohio Agricultural Research and Development Center
In Partnership With Ohio State University Extension

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Department of Animal Sciences

Research and Reviews: Poultry and Swine

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Department of Animal Sciences
Ohio Agricultural Research and Development Center
Ohio State University Extension
The Ohio State University

ANIMAL SCIENCES

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Foreword

The Ohio State University Department of Animal Sciences has a rich tradition and has made many contributions to food-animal agriculture in the state of Ohio and beyond. With the dramatic changes that are occurring in all segments of food-animal agriculture, we are challenged to be at the cutting edge to best serve society.

The department is in the process of developing a strategic plan to shape the areas of extension/outreach/engagement, research, and teaching on which the department will focus in the future. The department has accomplished much in the strategic planning that has already been performed. Working with the Animal Sciences Visionary Team, composed of faculty members and industrial partners, however, we will finalize our strategic plan. Without question, there will be continued focus in this department on production efficiency of food animals (cattle, pigs, poultry, and sheep). Other areas that the department is giving strong consideration to are socially responsible and environmentally compatible food-animal management, safety and quality of food-animal products, and understanding the regulation of genes to help in controlling the efficiency of production and the quality and safety of the products that we harvest from food-producing animals. Our department is in the process of hiring faculty members to pursue some of these new areas of study. Indeed, we in land-grant institutions are facing the same challenges that the production segments of agriculture are facing — we need to make wise changes in order to maintain our productivity and efficiency. We also need to remain flexible, so that we can continue to make changes in the future to best serve our stakeholders. The extent to which we continue to serve as we have in the past and to what extent we focus on new areas will be the primary goal of the Animal Sciences Visionary Team. New faces will continue to appear in the *Research and Reviews*, along with changes in the descriptions of the activities of existing faculty so that we can make the changes to better serve our stakeholders.

A question we are frequently asked is to what extent do we intend to expand the scope of the Department of Animal Sciences beyond food-animal production. We have had programs in the equine science area for several years and plans are to continue this program. To what extent we expand into other areas, whether it be new areas of food-animal production or other areas such as companion animal sciences, will be a topic of discussion by our Animal Sciences Visionary Team. Regardless of the programs on which we choose to focus or expand, significant changes will be occurring in the Department of Animal Sciences at The Ohio State University. Whatever we do in changing the department, we want to have a program that is very strong in educating the many students who choose our department to pursue their education. Several of the new faculty members will focus on serving students. We believe that if we have faculty who are dedicated to providing high-quality educational opportunities to our students that we will continue to have a strong student body in the Department of Animal Sciences at The Ohio State University.

My first two months at Ohio State have been very stimulating. I am enjoying working with the faculty and staff and having more students on campus for the academic year. My focus will continue to be on the faculty and the staff members of this department because, in order to serve our students and stakeholders in an efficient and effective fashion, we must have faculty and staff members who are functioning in an effective and efficient manner. My secondary focus is on leading the Animal Science Building Initiative to develop facilities that are of similar quality to those of our neighboring land-grant institutions. I look forward to working with the citizens in the state of Ohio in our educational and building initiatives.

James E. Kinder
Chair

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Faculty

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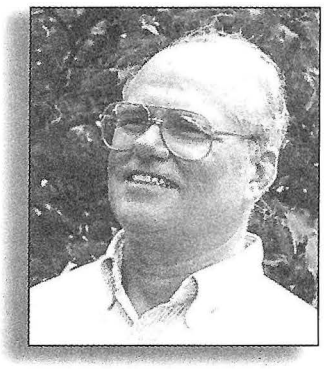
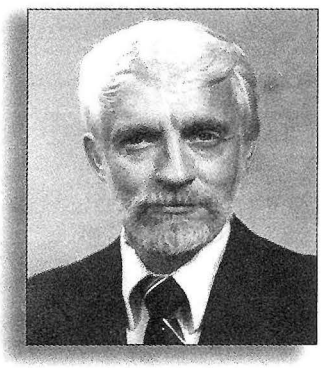
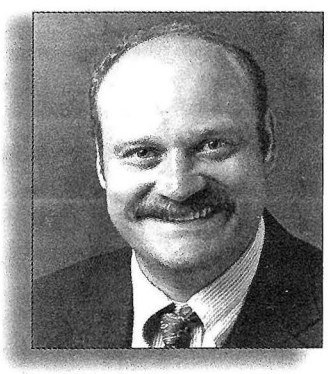


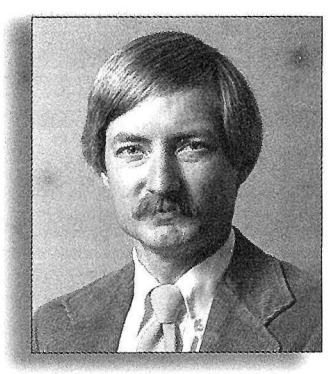
F. R. Allaire, Professor, Columbus. Offers a capstone course where students, working in teams, learn to manage change that serves a client's vision. Works with the Agroecosystems Management Program to inform and facilitate knowledge management networks to support farmers in their pursuit of systemic change in their respective enterprises and communities. Works to have animals add value to community life and land.



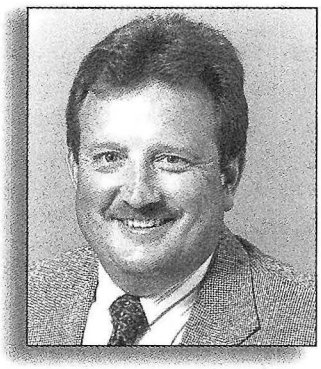
W. L. Bacon, Professor, Wooster. Dr. Bacon's main research focus is avian reproduction. The effects of environmental lighting on semen quality and quantity, and the control of photorefractoryness in the male turkey are being studied. The effects of environmental lighting on circulating hormones at the initiation of photostimulation, and the ovarian-pituitary relationship during the ovulatory surge in the female turkey are also being studied. In female Japanese quail, the effect of yolk precursor lipoprotein concentration on lipid composition of the precursor, and metabolic rate of the precursor is determined.



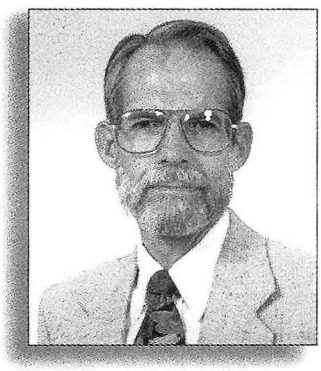
S. L. Boyles, Associate Professor, Columbus. Dr. Boyles is responsible for the state beef cattle education outreach program. His Extension program includes coordinating the activities of the OSU Extension Beef Team and conducting local education workshops. Dr. Boyles' research program emphasizes improved forage utilization through grazing strategies and hay storage systems. Dr. Boyles also is working with commodity organizations on improving beef cattle marketing programs.



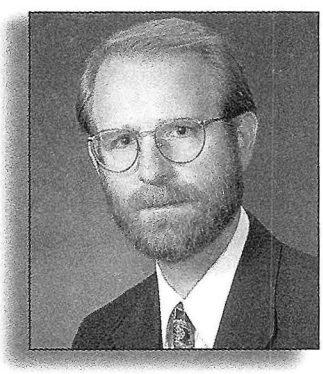
M. E. Davis, Professor, Columbus. Dr. Davis' teaching duties include: Data Analysis and Interpretation for Decision Making (AS 260), Principles of Animal Improvement (AS 320), Research Methods in Animal Genetics I and II (AS 820.02 and 820.04). Research responsibilities include genetics research with the beef herd at the Eastern Ohio Resource Development Center and emphasize studies of postweaning feed conversion, twinning, selection for IGF-I, and marker/QTL associations for growth and body composition in beef cattle. Dr. Davis also is Director of the Animal Genetics Lab, which conducts blood and NA typing for parentage verification for several of the major beef cattle breed associations.



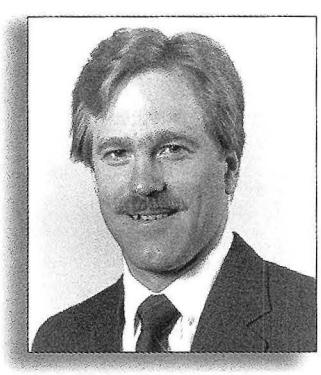
M. L. Day, Associate Professor, Columbus. Dr. Day's research program in reproductive physiology emphasizes the study of puberty, postpartum reproduction, and estrous synchronization in cattle. He teaches the Beef Production and Management and Introductory Animal Sciences courses and advanced reproduction for graduate students. He serves as Faculty Supervisor of the OSU Beef Center.



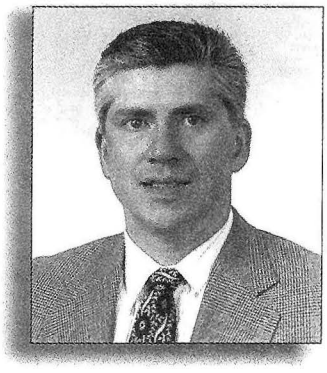
B. A. Dehority, Professor, Wooster. Dr. Dehority's teaching responsibilities include a course in Rumen Microbiology taught every other year during the summer quarter at Wooster, and he advises graduate students. His research interests are in the area of rumen microbiology, including the role of fungi in the rumen, development of MPN procedures for counting rumen bacteria and fungi, the isolation and characterization of rumen bacteria responsible for the breakdown of forage structural carbohydrates, factors affecting protozoal numbers, and specificity of gastrointestinal protozoa, as well as various other specific studies in rumen microbiology.



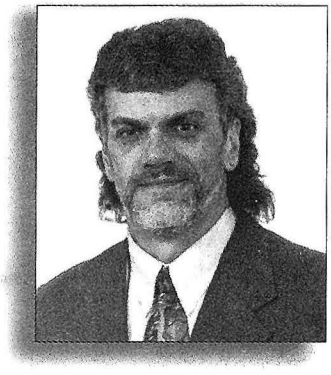
M. L. Eastridge, Professor, Columbus. Dr. Eastridge has Extension and research responsibilities in dairy cattle nutrition and serves as the Coordinator of the Extension Program in Animal Sciences and faculty supervisor for the Waterman dairy facility in Columbus. He conducts educational programs in the area of nutrition. Research includes the impact of fats and feed additives on animal performance and milk composition, and study of optimum fiber in diets for lactating cows. Teaching responsibilities include advising graduate students and co-teaching and serving as leader for an applied dairy nutrition course designed primarily for veterinary students.



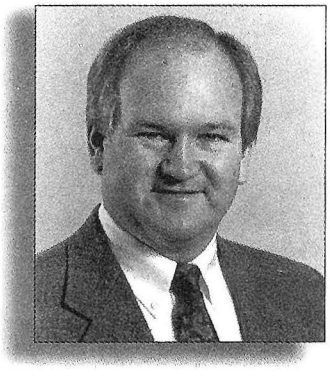
J. L. Firkins, Associate Professor, Columbus. Dr. Firkins' research activities include evaluation of by-products as fiber sources and fat and protein sources for dairy cattle. He studies how these feeds and feeding combinations affect the site of nutrient digestion and efficiency of microbial protein synthesis in the rumen of cattle. Dr. Firkins teaches AS 330, Principles of Animal Nutrition; AS 530, Comparative Animal Nutrition; and AS 730.02, Research Techniques in Animal Nutrition.



F. L. Fluharty, Research Scientist, Wooster. Dr. Fluharty's responsibilities include conducting research in beef cattle and sheep nutrition. His primary research areas are determining the effects of energy and protein intake on animal growth and carcass composition and the nutritional requirements of stressed feeder calves. His research includes work with ruminal microbiology and digestion, as well as cattle and sheep performance studies. He currently is conducting research to determine the effects of nutrition and genetics on animal growth and meat tenderness and the effects of early-weaning beef calves on subsequent feedlot performance and carcass composition. He also teaches AS 540 (Feedlot Management).

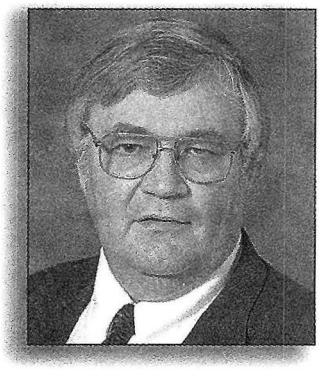


J. S. Hogan, Associate Professor, Wooster. Dr. Hogan's research is in the area of bovine mastitis: hygiene procedures to reduce bovine intramammary infection; relationships among normal and transit teat skin bacterial flora; and milk quality enhancement. He also conducts research relative to the development of a mastitis vaccine, and he teaches the undergraduate lactation course.

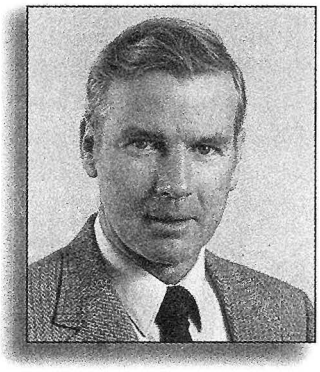


K. M. Irvin, Professor, Columbus. Dr. Irvin's research focuses on swine genetics. Primary consideration is made to the combination of population genetics and molecular genetics. Teaching responsibilities include Principles of Genetic Improvement; Application of Genetic Improvement to Swine; Population Genetics I and II; Advanced Swine Production; Current Issues in Animal Sciences, Capstone and Third Writing Course; Seminars; Independent Studies; and Internships. Extension functions include presentations, allied industry and producer interactions.

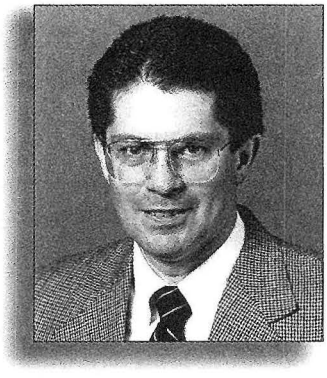
C. Johnston, Professor, Wooster. Dr. Johnston's research interests are in the areas of modification of non-milk ingredients for inclusion in milk replacers for cattle and sheep, and dietary macromolecular absorption by cattle and sheep.



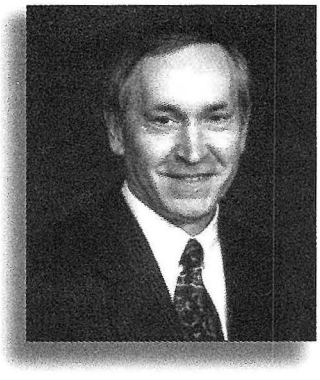
J. E. Kinder, Professor and Chair, Columbus. Dr. Kinder, along with the Associate Chair, Dr. Joy Pate, provides the leadership for administering the various programs in the Department of Animal Sciences. Dr. Kinder also supervises graduate students and conducts research in the area of hormonal regulation of the reproductive function. The focus of his research program is on hormonal regulation of sexual maturation and the reproductive cycle of cattle. He also has an active research program in developing practical technologies to control reproductive cycles of cattle.



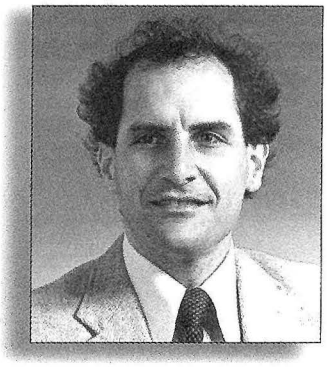
R. C. Kline, Associate Professor, Columbus. Dr. Kline's responsibilities include teaching the horse courses: AS 201, AS 271, and AS 541. His Extension activities include conducting eleven state-wide events each year for the 4-H Program, writing horse materials for both youth and adult programs, and answering the daily requests for information from the horse industry. He oversees the University horse herd and its use for classes and research. Present research involves equine behavior and reproductive physiology in horses.



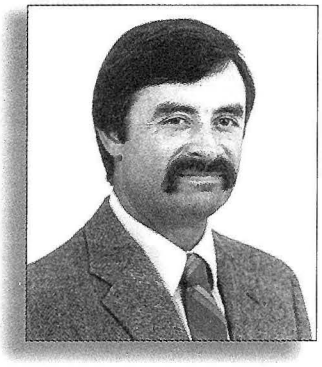
C. L. Knipe, Associate Professor, Columbus. Primary responsibilities include processed meat extension activities for the Ohio meat industry. He is also involved in research and teaching and has a joint appointment with the Department of Food Science and Technology. In addition to providing technical assistance to small and large companies, within Ohio and nationwide, his extension activities have focused on Hazard Analysis and Critical Control Point (HACCP) training and implementation assistance for Ohio meat plants. Dr. Knipe's research interests are identification of processing methods which optimize the functional quality of pork, identification of processing procedures which limit shelf-life and/or safety of meat products, including shelf-life determination of such products, and maximizing the functionality of high-collagen meat raw materials. He advises graduate students in Meat Science and teaches Animal Science 550 (Meat Processing).



J. D. Latshaw, Professor, Columbus. Dr. Latshaw's teaching responsibilities include an introductory course in animal nutrition and one in poultry science. Also, he teaches half of the second nutrition course and half of a course combining nutrition and physiology in support of reproduction. His research interests include documenting all nutrient deficiencies and excesses in broiler chicks and examining the use of energy by birds.



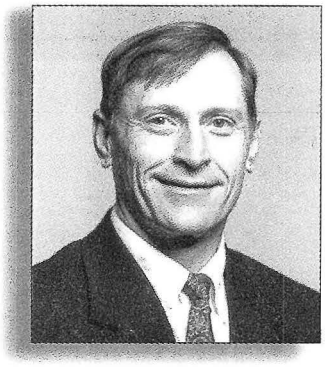
M. S. Lilburn, Associate Professor, Wooster. Dr. Lilburn's research focuses on different aspects of avian nutrition and avian embryonic development. His teaching responsibilities are AS 830.05, a graduate vitamins course, and AS 830.03, a graduate course in proteins. Dr. Lilburn also advises students on the graduate level.



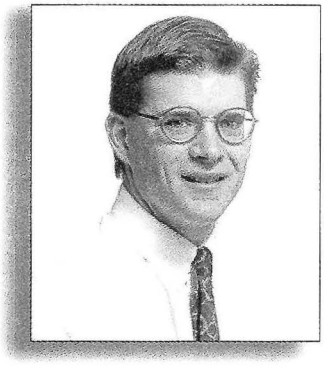
S. C. Loerch, Professor, Wooster. Dr. Loerch's primary research responsibility is in beef cattle nutrition, including effects of controlling intake on feedlot performance and proportion of carcass lean and fat, use of extended grazing and corn as alternative feeds for wintering beef cows, and nutritional strategies for stressed feeder calves. He supervises the OARDC Beef Center and the cow herd at the North Appalachian Experimental Watershed Branch in Coshocton. He teaches an undergraduate practical nutrition course and a graduate-level advanced ruminant nutrition course.

D. C. Mahan, Professor, Columbus. Dr. Mahan's research responsibilities involve evaluating the nutritional requirements and feeding programs of swine at various stages of production, with primary emphasis on the sow and weanling pig. Nutritional areas of investigation include vitamin E and selenium, sodium and chloride requirements of young pigs, dietary protein and energy levels for the gestating and lactating sow, and the evaluation of carbohydrate and energy sources for the weanling pig. He teaches undergraduate courses in Animal Growth and Development and Feeds and Feeding and a graduate course in Mineral Nutrition.

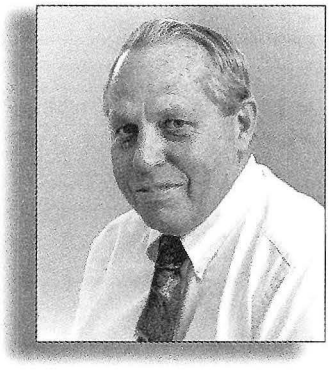
K. E. McClure, Assistant Professor, Wooster. Dr. McClure's research interest is ruminant nutrition with emphasis on forage utilization. Research efforts include grazing experiments with legumes and cool-season grasses using lambs. The objective is meat production that maximizes lean and minimizes fat for health-conscious consumers. Strategies that involve grazing and/or comparative supplemental energy, protein, vitamins, and minerals that are adaptable to the sheep enterprise are a primary objective. Emphasis is also directed to the use of the corn plant and other forages in the basal diet of the ewe flock and breeding rams to economically meet their nutritional requirements. Extension participation includes phone consultations, forage-related farm visits, and meeting with producer groups.



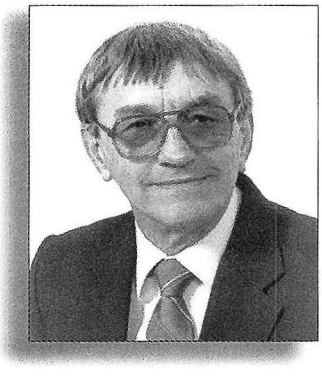
D. L. Meeker, Associate Professor, Columbus. Dr. Meeker's primary responsibilities are in Extension, although he is also involved in research and teaching. He is Coordinator of the Ohio Pork Industry Center, which coordinates expertise from various disciplines to facilitate the profitable and environmentally responsible production of wholesome pork. The Center is an outreach activity of OSU Extension. Dr. Meeker's research interests include swine genetics, particularly genetic effects on muscle quality. He teaches Animal Sciences 643, Advanced Swine Production.



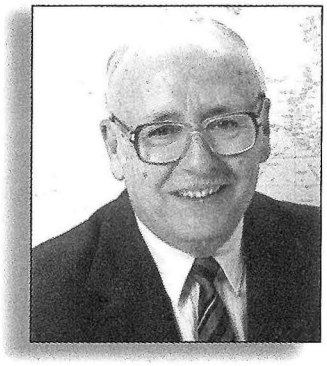
S. J. Moeller, Assistant Professor, Columbus. Dr. Moeller's Extension responsibilities include swine production, management, and genetics/breeding. He works as co-leader of the OSU Swine Educators Team to assist with in-service training and development of comprehensive statewide educational and technology transfer programs important to the Ohio swine industry. His primary research interests are in swine genetics and production management strategies. Teaching responsibilities include Swine Production and Advanced Swine Production.



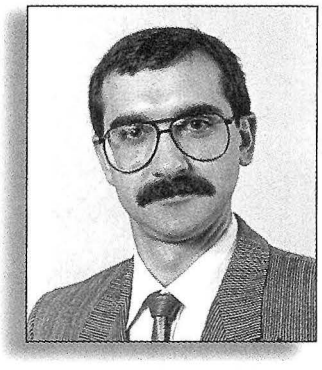
F. V. Muir, Professor, Wooster. Dr. Muir's primary outreach interests are in the areas of the management of layers and broilers, especially the application of computers in the production of eggs and poultry meat. The use of computers to integrate egg production or growth data, feed consumption, poultry house environment, feed formulation, and record keeping are important considerations in remaining competitive. Course responsibility — Commercial Poultry Management.



K. E. Nestor, Professor, Wooster. Dr. Nestor's research interests include population genetics of turkeys and Japanese quail, genetics of disease resistance in the turkey, genetic relationships between growth and reproduction, and genetics of leg strength in the turkey. He advises graduate students and is host to several visiting scholars. Dr. Nestor is a Fellow of the Poultry Science Association and a member of Gamma Sigma Delta.



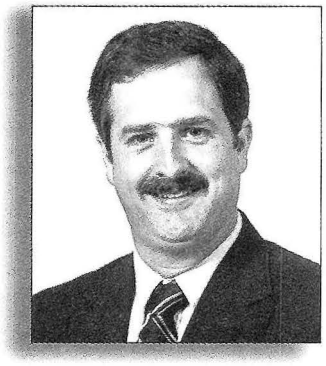
H. W. Ockerman, Professor, Columbus. Dr. Ockerman's teaching responsibilities include Advanced Meat Technology, Laboratory Analysis of Meat Products, Quality Control Interpretation, Global Food and Agriculture, Food in International Agriculture, and Meat Science Seminars, as well as internships and individual studies. He is also involved in international education. His research programs include biochemistry, microbiology, processing, quality, food safety, shelf life, and economics of muscle tissue from slaughter to consumption in all species. Extension duties include short courses, consulting, legal evaluation, and trouble-shooting industry concerns.



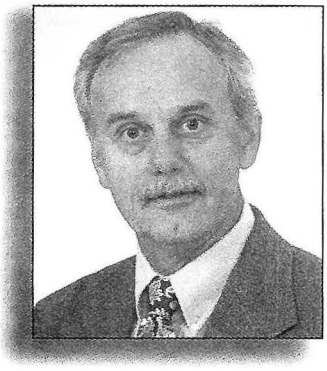
J. S. Ottobre, Professor, Columbus. Dr. Ottobre's research is in the area of reproductive physiology. The primary focus of this research is the regulation of the function of the corpus luteum. He teaches Introductory Animal Science, Reproductive Physiology, and Advanced Reproductive Physiology. Dr. Ottobre has a joint appointment in the Department of Physiology in the College of Medicine.

D. L. Palmquist, Professor, Wooster. Dr. Palmquist's research is in the area of dairy cattle nutrition, including digestive physiology and nutrient utilization of high-energy diets, especially fats, and regulation of milk synthesis and composition. He teaches graduate courses in ruminant nutrition.

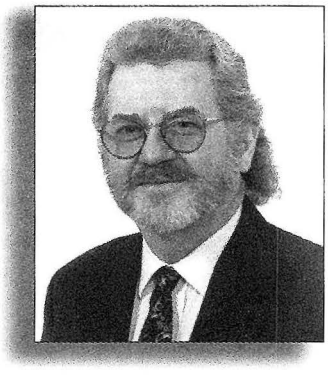
J. L. Pate, Professor and Associate Chair, Wooster. Dr. Pate is a reproductive physiologist specializing in the area of corpus luteum function. Primary research interests focus on the regulation of luteolysis, prostaglandin production by the corpus luteum, the interactions between the immune system and the reproductive system, and nutritional/metabolic effects on fertility. She teaches Physiology of Reproduction and Advanced Reproductive Endocrinology.



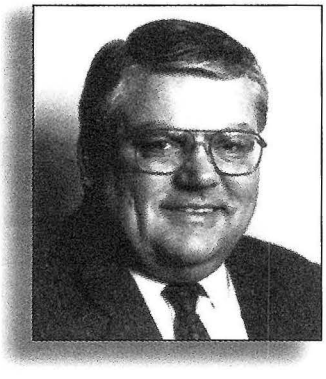
W. F. Pope, Professor, Columbus. Dr. Pope's primary research interests are in embryonic mortality in swine. Secondary investigations are examining factors affecting fertilization, estrous cycle control, uterine secretions, and isoforms of the estradiol receptor. His teaching responsibilities include the core physiology course (310) and reproductive physiology (410). Extension duties include working closely with commercial sheep producers through field days and site visits.



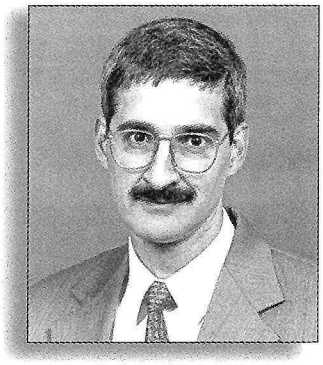
F. L. Schanbacher, Professor, Wooster. Dr. Schanbacher's research interests are in the areas of physiology, biochemistry, and molecular biology of bovine mammary development and milk protein synthesis. Studies are focused at whole animal, cellular, and molecular biology levels for synthesis and secretion of milk protein, mammary cellular growth and development, and growth regulation. He teaches the advanced course in Physiology of Lactation.



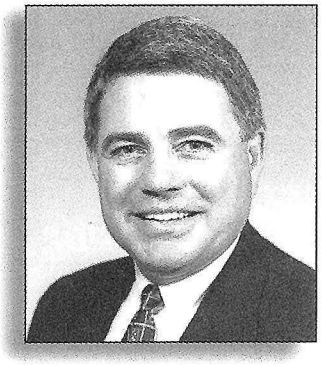
K. L. Smith, Professor, Wooster. Dr. Smith's research is in the area of diagnosis, therapy, and control of bovine mastitis in dairy herds; natural factors of disease resistance associated with the bovine mammary gland; and environmental and nutritional factors associated with increased mastitis in dairy herds. He advises numerous M.S. and Ph.D. students.



P. W. Spike, Associate Professor, Columbus. Dr. Spike has appointments in Extension and teaching, including Extension responsibilities in youth work (4-H and FFA), genetics, and management. His teaching duties include dairy cattle evaluation, dairy herd management, and dairy farm management. He also coaches the dairy cattle judging teams and advises the Buckeye Dairy Club.

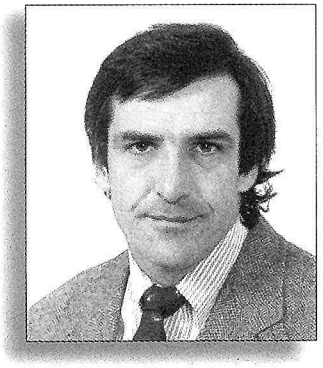


N. R. St-Pierre, Associate Professor, Columbus. Dr. St-Pierre specializes in the area of dairy farm management. Research interest is in the control function of management. Ongoing research projects are focused on quantitative methods for evaluating animal systems (production, reproduction, and mammary health); the value of milk urea nitrogen (MUN) as a nutrition management tool; reduction of nitrogen excretion by dairy cows and feed cost optimization and nutritional economics; and production risks and risk management for dairy farms. Extension programs focus on three inter-dependent areas: long-term strategic planning of dairy enterprises; production and financial benchmarks for evaluating short-, medium-, and long-term results; and nutritional management, herd structure, and cost control.

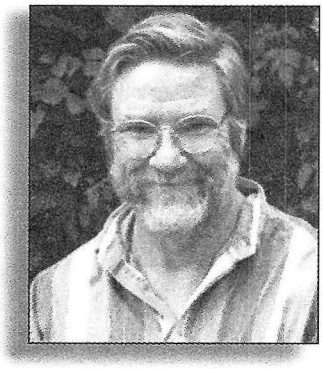


T. B. Turner, Assistant Professor, Columbus. Dr. Turner's primary research interest is beef cow performance, including milk production, preweaning calf performance, age at weaning, and matching feed resources to calving and weaning management. His teaching responsibilities include Introductory Animal Sciences, Livestock Selection and Evaluation, and Applied Beef Cattle Genetics, and he coaches the Intercollegiate Livestock Selection and Evaluation Team. Extension responsibilities include programs in beef cattle genetic improvement and in livestock selection and evaluation. He also advises undergraduate and graduate students.

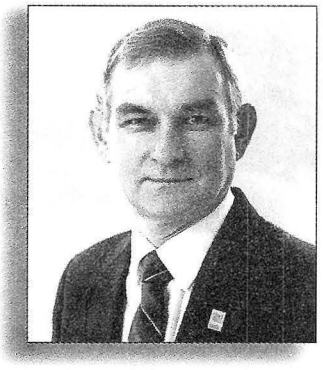
S. G. Velleman, Assistant Professor, Wooster. Dr. Velleman's research focuses on how the extracellular matrix influences skeletal muscle growth and function. She teaches AS 618, Early Embryonic Development in Support of Tissue Growth, Structure, and Function.



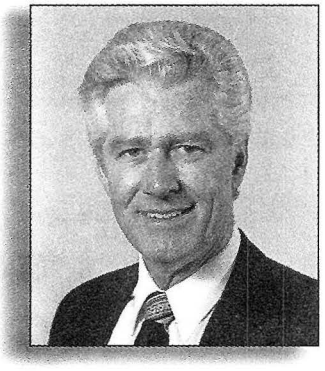
W. P. Weiss, Professor, Wooster. Dr. Weiss' research is in the area of forage utilization, feed evaluation, and nutritional factors affecting cow health. Extension duties include working with the Ohio Dairy Ration Program and teaching dairy nutrition short courses.



M. P. Wick, Assistant Professor, Columbus. Dr. Wick's research focuses on the role of sarcomeric proteins in the mechanisms controlling skeletal muscle growth and development and meat quality. Teaching responsibilities include AS 355.01, Principles of Meat Science; AS 620, Applied Animal Molecular Genetics; and AS 868, Molecular Biology Techniques.



L. B. Willett, Professor, Wooster. Dr. Willett's research interests are in the area of food, feed, and animal safety, which is the study of the movement and effects of hazardous or toxic materials in food-producing animals. He also studies the physiological adaptive changes that occur in calves immediately after birth. His teaching responsibilities are in the graduate toxicology courses, and he advises graduate students in physiology and toxicology. Dr. Willett also advises independent study students in a collaborative effort with the College of Wooster.



D. L. Zartman, Professor, Columbus. Dr. Zartman's areas of interest and expertise include biotelemetry to improve animal performance through increased physiological data for improved decision making or through modification of cellular processes. He advocates intensive grazing and seasonal dairying research and also works in genetics, cell culture, and reproductive physiology. Classes taught include animal welfare/rights issues and management intensive grazing.

Effect of Selection for Increased Body Weight on Mitogenic Responses in Turkeys

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Abstract

Mitogenic responses were examined for purified peripheral blood mononuclear cells (PBMC) and whole blood from individuals in a line (F) of turkeys selected for increased 16-week body weight and its corresponding randombred control (RBC2). The PBMC were isolated by centrifugation over Histopaque-1077 density gradient and tested for mitogenic responses to concanavalin A (ConA; 25 μg/mL) and phytohemagglutinin M (PHA-M; 100 μg/mL). For the whole blood assay, six-week-old poults from both lines were injected with inactivated *Pasteurella multocida*. Heparinized blood samples were collected prior to injection (0 days) and at 2, 4, 7, and 14 days postinjection. The diluted whole blood was then tested for the mitogenic responses to Con A (25 μg/mL) and PHA-M (25 μg/mL). The cultures were then pulsed with ^{3}H-thymidine, and incorporation was measured using a liquid scintillation counter.

There was a line difference in the mitogenic responses to ConA for PBMC and whole blood assays, but no line difference was observed in the response to PHA-M for both assays. For the purified PBMC assay, the F line had a lower response than its randombred control line ($P \leq 0.05$) to Con A expressed as either cpm or a stimulation index (SI; ratio of cpm for stimulated cells to the cpm for unstimulated cells). For the whole blood assay, the F line had generally lower SI values in the responses to Con A than the RBC2 line with differences being significant at 0 and 2 days postinjection ($P \leq 0.01$) and at 14 days postinjection ($P \leq 0.05$). Genetic selection for increased body weight may have affected the lymphoblastogenic potential of Line F that could affect disease resistance.

Introduction

Host resistance to pathogens is complicated and involves both specific and nonspecific resistant factors. The humoral immune response is the principle specific immunity against extracellular bacteria, whereas the cell-mediated immunity plays a major role in the responses against intracellular bacteria and viruses (Abbas *et al.*, 1994). Different genetic backgrounds in individuals may vary in these facets of host immunity and influence resistance to infectious diseases.

Previous reports showed that a line (F) of turkeys selected for increased 16-week body weight was more susceptible to erysipelas, fowl cholera, and Newcastle disease than its parental randombred control line (RBC2) (Saif *et al.*, 1984; Sacco *et al.*, 1991; Tsai *et al.*, 1992; Nestor *et al.*, 1996b). However, the antibody titers detected with a hemagglutination inhibition test were higher in the surviving F-line turkeys than those of the RBC2 line in a challenge experiment with Newcastle disease virus (Tsai *et al.*, 1992). In addition, the F line had higher antibody responses to Newcastle disease virus vaccines as measured by ELISA (Sacco *et al.*, 1994). These results suggest that there was no positive correlation between resistance to a specific disease and humoral response in these turkeys. Therefore, it is appropriate to suggest that other immune mechanisms, such as cell-mediated

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immunity, may be contributing to the explanation of the susceptibility of the F line. The mitogenic response assay is a practical measure of cell-mediated immunity. The purpose of the present study was to examine the mitogenic responses to plant lectins in the F and RBC2 lines of turkeys.

Materials and Methods

Turkeys

Six-week-old poults from each line were used in the experiments. The RBC2 line was a randombred control line initiated in 1966 (Nestor, 1977a). The F line was a subline of RBC2 selected for increased 16-week body weight (Nestor, 1977b, 1984; Nestor *et al.*, 1996a). The inbreeding coefficient of the RBC2 and F lines was 12.6 and 27.1%, respectively, at the time of the study. The birds were provided feed (Naber and Touchburn, 1970) and water for *ad libitum* consumption. The lines were grown intermingled in each experiment.

Mitogenic Responses

For the mitogenic responses of purified peripheral blood mononuclear cells (PBMC), 2 mL of heparinized blood were collected from 10 RBC2 and 14 F line individuals of equal numbers of males and females. The PBMC were isolated by centrifugation over Histopaque-1077 (Sigma Chemical Co., St. Louis, MO 63178-9916) density gradient, and cell viability was assessed by the means of trypan blue dye exclusion. Cells were then enumerated and adjusted to 1 x 10^7 cell /mL in RPMI 1640 medium (Sigma Chemical Co., St. Louis, MO 63178-9916) containing penicillin (100 units/mL), streptomycin (100 μg/mL), and 5% complement-inactivated (56°C for 30 min) chicken serum (Sigma Chemical Co., St. Louis, MO 63178-9916). Cells were cultured at a concentration of 1 x 10^6 cells in 200 uL/well in 96-well culture plates under stimulation in triplicate *in vitro* with either concanavalin A (Con A; Sigma Chemical Co., St. Louis, MO 63178-9916) (25 μg/mL), or phytohemagglutinin-M (PHA-M; Boehringer Mannheim Corporation, Indianapolis, IN 46250-0414) (100 μg/mL), or with medium alone. Cells were incubated for 52 hours at 39°C in a humidified incubator with 5% CO_2, then pulsed with 20 μL of ^{3}H-thymidine (1 μCi, 6.7 Ci/mmol) for 16 hours. Cells were harvested on glass fiber filters by using a cell harvester (Skatron, Inc., Sterline, VA 20166). The ^{3}H-thymidine incorporation was measured using a liquid scintillation counter.

A whole blood assay was carried out according to the methods described by Sharma and Belzer (1992), with some modification. Briefly, 14 turkeys from each line were subcutaneously injected with 1 mL of formaldehyde-inactivated *Pasteurella multocida* (1.2 x 10^7 bacteria/mL) at six weeks of age. Heparinized blood samples were collected before injection (0 days) and at 2, 4, 7, and 14 days postinjection. Whole blood cells were diluted 1:20 with RPMI-1640 containing penicillin (100 units/mL), streptomycin (100 μg/mL), 5 x 10^{-5} M 2-mercaptoethanol, and 2% pooled and heat-inactivated (56°C for 30 min) turkey serum. One hundred microliters of diluted blood was dispensed into the 96-well flat-bottomed culture plates which contained 100 μL of Con A (50 ug/mL), or PHA-M (50 ug/mL), or medium alone in triplicate. Cultures were grown at 40°C for 52 hours in 5% CO_2 humidified incubator. The cell cultures were then labeled with 20 μL of ^{3}H-thymidine (1 μCi) for an additional 16 hours. Cells were lysed by repeated freezing (at -20°C) and thawing three times and were harvested for liquid scintillation counting as noted previously.

Statistical Analysis

Means of counts per minute were calculated for triplicate cultures. Results for each PBMC sample were expressed as the mean counts per minute for cells stimulated by ConA or PHA-M minus the mean counts per minute of background. A stimulation index (SI) was obtained by dividing the average counts per minute from stimulated cells by the average counts per minute from unstimulated cells. An ANOVA was used to test for the significant differences in the mitogenic responses between lines and sexes using the General Linear Model procedure of SAS® (SAS Institute, 1988). All data were expressed as the least squares mean ± SEM.

Results

There was no significant sex effect on mitogenic responses for either purified PBMC or whole blood. However, there was a significant line effect for Con A but not for PHA-M stimulation of PBMC (Table 1). The RBC2 line had significantly higher responses to Con A when the results were expressed either as counts per minute or SI ($P \leq 0.05$).

Table 1. Mitogenic Responses to Concanavalin (Con A) and Phytohemagglutinin-M (PHA-M) of Peripheral Blood Mononuclear Cells from the RBC2 and F Lines of Turkeys (Least Squares Mean ± SEM).

Mitogen	Line[1]	
	RBC2	F
	Counts per minute x 10^{-3}	
Con A	23.9 ± 3.5[a]	10.9 ± 2.9[b]
PHA-M	9.9 ± 2.7	14.4 ± 2.2
	Stimulation Index (SI)[2]	
Con A	29.6 ± 5.8[a]	8.3 ± 4.9[b]
PHA-M	14.4 ± 3.7	11.8 ± 3.1

[a,b] Means within a row with no common superscript differ significantly ($P \leq 0.05$).

[1] RBC2 = a randombred control line and F = a subline of the RBC2 line selected for increased 16-week body weight. The number of samples was 10 and 14 for Lines RBC2 and F, respectively.

[2] SI = ratio of the cpm for stimulated cultures to the cpm for unstimulated cultures.

For the mitogenic responses tested with the whole blood assay, the results showed that mitogenic responses to Con A expressed as SI were greater in the RBC2 line than in the F line with differences being significant at 0 and 2 days postinjection (both $P \leq 0.01$) and at 14 days postinjection ($P \leq 0.05$). The SI values from both lines peaked at 2 days postinjection. However, there was no significant difference in the responses to PHA-M between the two lines.

Discussion

The mitogenic response to plant lectins is conventionally used to measure the cell-mediated immunity in mammals and aves. The Con A and PHA mitogens stimulate T lymphocytes (Toivanen and Toivanen, 1973; Hovi *et al.*, 1978) by indirectly cross-linking the T cell receptor complex (Abbas *et al.*, 1994). Compared with the assays using purified PBMC, whole blood assays are easy, rapid, more valid, and a true measure of cellular immune competence, and therefore suitable for monitoring the functional capabilities of immune cells in avian flocks (Lee, 1978; Sharma and Belzer, 1992; Talebi *et al.*, 1995). However, there is individual variation in the whole blood assay (Sharma and Belzeer, 1992) so both the purified PMBC and whole blood assays were used in the present study.

In separate studies, the counts per minute and SI of purified PBMC were lower than those of cells prepared from buffy coat possibly because the preparation of the lymphocytes through Histopaque-1077 caused lower responses to mitogens (unpublished data). It has been reported that Ficoll (sodium diatrizoate is a major component and also is the main component of Histopaque-1077) may have some inhibitory effect on the mitogenic stimulation of lymphocytes (Lee, 1974; Maheswaran *et al.*, 1975). In addition, the ratio of cell types may vary with different procedures of cell preparation. Erf and Smyth (1996) reported that PBMC prepared through Histopaque-1077 may contain more thrombocytes and monocytes in addition to lymphocytes, whereas cells obtained from buffy coats by slow-speed centrifugation may be rich in lymphocytes and some contamination with erythrocytes and thrombocytes, but contain no monocytes. However, there was less variation among individuals within lines in the mitogenic responses with purified PBMC than with buffy coat cells (unpublished data). The mitogenic responses are also affected by many other parameters, such as the storage time (Raj *et al.*, 1997), the enhancement by erythrocytes in the cultures (Powell, 1980), the suppression by monocytes (Vainio and Ratcliffe, 1984; Schaefer *et al.*, 1985), serum sources and concentrations, incubation temperature, and length of assay (Lee, 1974, 1978; Maheswaran *et al.*, 1975; Sharma and Belzer, 1992; Talebi *et al.*, 1995).

The results herein demonstrated that the F line had lower responses to Con A using either purified PBMC or whole blood than the RBC2 line, but there was no line difference in the responses to PHA-M. In the subsequent challenge experiment with virulent *P. multocida*, it was observed that the poults that died earlier in the F line usually had smaller SI values for Con A-stimulated PBMC (data not shown). The Pearson correlation coefficient was 0.40 (P = 0.052) between SI for Con A and the number of days to death post-injection with *P. multocida*. The correlation coefficient between the SI for PHA and days to death was not significant. The mortality following challenge with *P. multocida* was 30 and 85.7% for the RBC2 and F line, respectively.

The results indicated that mitogenic responses to Con A may be related to resistance to specific diseases. The different responses to Con A in the two lines of turkeys may be due to genetic variation. In the present study, there was no significant statistical difference in mitogenic responses to PHA-M between two lines. Different gene systems may mediate blood lymphocyte responsiveness to Con A and PHA in chickens (Miggiano *et al.*, 1976; Morrow and Abplanalp, 1981; Fredericksen and Silmour, 1983). In addition, the mitogenic response to Con A is suggested to be controlled by at least two genes — a non-major histocompatibility complex-associated gene (ConA1) and a major histocompatibility complex-associated gene (ConA2) (Morrow and Abplanalp, 1981; Knudtson *et al.*, 1990).

Previous results have shown that the F line was susceptible to several infectious diseases. It was not possible to correlate the susceptibility of the F line to Newcastle disease with antibody response (Tsai *et al.*, 1992; Sacco *et al.*, 1994), or with known changes in the frequency of the MHC Class II haplotypes for both *P. multocida* and Newcastle disease virus (Nestor *et al.*, 1996c). In highly inbred chicken lines, Knudtson *et al.* (1990) found that the level of inteleukin-2-like activity was associated with the level of mitogenic response to Con A. Selection for increased body weight in the F line may have resulted in changes of gene frequency for genes controlling mitogenic responses to Con A, cytokines, and other facets of cell-mediated immunity, therefore affecting disease resistance. The mitogenic response to Con A in whole blood assay may have a potential for use as an indicator for genetic selection for improved disease resistance.

References

Abbas, A. K., A. H. Lichtman, and J. S. Pober. 1994. *Cellular and Molecular Immunology.* 2nd Ed. W. B. Saunderns Co., Philadelphia, Pa.

Erf, G. F. and J. R. Smyth Jr. 1996. Alterations in blood leukocyte populations in Smyth line chickens with autoimmune vitiligo. *Poultry Sci.* 75:351-356.

Fredericksen, T. L. and D. G. Gilmour. 1983. Ontogeny of Con A and PHA responses of chicken blood cells in MHC-compatible lines 6_3 and 7_2. *J. Immunol.* 130:2528-2533.

Hovi, T., J. Suni, L. Hortling, and A. Vaheri. 1978. Stimulation of chicken lymphocytes by T and B cell mitogens. *Cell. Immunol.* 39:70-78.

Knudtson, K. L., M. G. Kaiser, and S. J. Lamont. 1990. Genetic control of interleukin-2-like activity is distinct from that of mitogenic response in chickens. *Poultry Sci.* 69:65-71.

Lee, L. F. 1974. In vitro assay of mitogen stimulation of avian peripheral lymphocytes. *Avian Dis.* 18:602-609.

Lee, L. F. 1978. Chicken lymphocyte stimulation by mitogens. A microassay with whole blood culture. *Avian Dis.* 22:296-307.

Maheswaran, S. K., E. S. Thies, and C. Greimann. 1975. A micromethod for evaluating turkey lymphocyte responses to phytomitogens. *Am. J. Vet. Res.* 36:1397-1400.

Miggiano, H., M. North, A. Buder, and J. R. L. Pink. 1976. Genetic control of the response of chicken leucocytes to a T-cell mitogen. *Nature* 263:61-63.

Morrow, P. R. and H. Abplanalp. 1981. Genetic control of T-lymphocyte mitogenesis in chickens. *Immunogenetics* 13:189-200.

Naber, E. C. and S. P. Touchburn. 1970. *Ohio Poultry Rations.* Ohio Cooperative Extension Service Bulletin 343. The Ohio State University, Columbus, Ohio.

Nestor, K. E. 1977a. The stability of two randombred control populations of turkeys. *Poultry Sci.* 56:54-57.

Nestor, K. E. 1977b. Genetics of growth and reproduction in the turkey. 5. Selection for increased body weight alone and in combination with increased egg production. *Poultry Sci.* 56:337-347.

Nestor, K. E. 1984. Genetics of growth and reproduction in the turkey. 9. Long-term selection for increased 16-week body weight. *Poultry Sci.* 63:2114-2122.

Nestor, K. E., D. O. Noble, J. Zhu, and Y. Moritsu. 1996a. Direct and correlated responses to long-term selection for increased body weight and egg production in turkeys. *Poultry Sci.* 75:1180-1191.

Nestor, K. E., Y. M. Saif, J. Zhu, and D. O. Noble. 1996b. Research Notes: Influence of growth selection in turkeys on resistance to *Pasteurella multocida*. *Poultry Sci.* 75:1161-1163.

Nestor, K. E., Y. M. Saif, J. Zhu, D. O. Noble, and R. A. Patterson. 1996c. The influence of major histocompatibility complex genotypes on resistance to *Pasteurella multocida* and Newcastle disease virus in turkeys. *Poultry Sci.* 75:29-33.

Parker, C. W. 1987. Biochemical changes taking place in mitogen-treated lymphocytes. *Methods Enzymol.* 150:29-31.

Powell, P. C. 1980. The influence of erythrocytes on the stimulation of chickens by phytohemagglutinin. *Avian Pathol.* 9:465-470.

Raj, G. D., R. C. Jones, and C. E. Savage. 1997. Effect of storage of chicken and turkey blood on the lymphocyte response to concanavalin A and pokeweed mitogen. *Vet. Immunol. Immunopathol.* 56:353-362.

Sacco, R. E., K. E. Nestor, Y. M. Saif, H. J. Tsai, and R. A. Patterson. 1994. Effect of genetic selection for increased body weight and sex of poults on antibody response of turkeys to Newcastle disease virus and *Pasteurella multocida* vaccines. *Avian Dis.* 38:33-36.

Sacco, R. E., Y. M. Saif, K. E. Nestor, N. B. Anthony, D. A. Emmerson, and R. N. Dearth. 1991. Genetic variation in resistance of turkeys to experimental challenge with *Pasteurella multocida*. *Avian Dis.* 35:950-954.

Saif, Y. M., K. E. Nestor, R. N. Dearth, and P. A. Renner. 1984. Case report — Possible genetic variation in resistance of turkeys to erysipelas and fowl cholera. *Avian Dis.* 28:770-773.

SAS Institute. 1988. *SAS^7/STAT User's Guide.* SAS Institute, Inc., Cary, N.C.

Schaefer, A. E., A. R. Scafuri, T. L. Fredericksen, and D. G. Gilmour. 1985. Strong suppression by monocytes of T-cell mitogens in chicken peripheral blood leukocytes. *J. Immunol.* 135:1652-1660.

Sharma, J. M., and S. W. Belzer. 1992. Blastogenic response of whole blood cells of turkeys to a T-cell mitogen. *Dev. Comp. Immunol.* 16:77-84.

Talebi, A., P. R. Torgerson, and G. Mulcahy. 1995. Optimal conditions for measurement of blastogenic responses of chickens to concanavalin A in whole blood assays. *Vet. Immunol. Immunolpathol.* 46:293-301.

Toivanen, P., and A. Toivanen. 1973. Selective activation of chicken T lymphocytes by concanavalin A. *J. Immunol.* 111:1602-1603.

Tsai, H. J., Y. M. Saif, K. E. Nestor, D. A. Emmerson, and R. A. Patterson. 1992. Genetic variation in resistance of turkeys to experimental infection with Newcastle disease virus. *Avian Dis.* 36:561-565.

Vainio, O., and M. J. H. Ratcliffe. 1984. Proliferation of chicken peripheral blood leucocytes in response to pokeweed mitogen is macrophage-dependent. *Cell. Immunol.* 85:235-241.

Changes in Genetic Parameters Over Thirty Generations of Selection for Increased Body Weight in Turkeys

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Abstract

A line (F) of turkeys was selected over 30 generations for increased 16-week body weight. The base population for the F line was a randombred control population (RBC2) that was maintained along with the F line without conscious selection. In order to study the effects of selection on genetic parameters, the effect of selection on selected and correlated traits in the F line was studied, whenever possible, at 10-generation intervals (1 to 10, 11 to 20, and 21 to 30) and over all generations (1 to 30). Values of the F line were expressed as deviations from the RBC2 line in order to remove yearly environmental variations.

Selection was effective in increasing 16-week body weight in the F line. Selection differentials based on the mean of selected parents minus mean of entire population (intended) and intended selection differentials weighted for number of offspring produced (actual) did not consistently differ, indicating that natural selection was not opposing artificial selection. The realized heritability (h^2; ratio of genetic variation to total variation in a trait) of 16-week body weight in the F line based on the linear regression of selection response on accumulated actual selection differentials declined with selection, and the decline appeared to be slightly different for males than females. For both sexes combined, the realized h^2 was 0.309 ± 0.022 (standard error), 0.268 ± 0.033, 0.242 ± 0.026, and 0.254 ± 0.007, respectively, for Generations 1 to 10, 11 to 20, 21 to 30, and 1 to 30.

Genetic increases in 16-week body weight in the F line were positively associated with body weight at other ages (8, 20, and 24 weeks of age and at 50% egg production), days from stimulatory lighting to production of the first egg, and egg weight and negatively associated with egg production, intensity of lay (maximum and average clutch length and rate of lay), and hatch of fertile eggs. There was no significant relationship of 16-week body weight and total days lost from broodiness or fertility. Genetic changes in some correlated traits were not consistent in all generation intervals studied, indicating that the genetic correlation between the selected trait (16-week body weight) and the correlated trait changed with selection.

Introduction

Heritability estimates (h^2) for body weight of turkeys during the growing period are generally large. Based on earlier literature, Nestor *et al.* (1967) reported that the unweighted averages of published h^2 estimates of body weight in selected populations were 0.40, 0.42, 0.43, and 0.36, respectively, for birds in the age groups 0 to 8, 9 to 16, 17 to 24, and greater than 24 weeks of age. Based on data from eight commercial flocks, Arthur and Abplanalp (1975) found that the h^2 of 18-week BW was 0.42. Using randombred control populations, h^2 estimates of body weight were high, ranging from 0.40 to 0.68 (McCartney, 1961; Nestor *et al.*, 1967; Havenstein *et al.*, 1988).

Results of earlier selection studies also suggested that h^2 of body weight was high. Abplanalp *et al.* (1963) found that the realized h^2 of 8- and 24-

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week body weight was 0.43 and 0.62, respectively. McCartney *et al.* (1968) observed that the realized h^2 of 8- and 24-week body weight was 0.44 and 0.39, respectively. Based on four generations of selection, Mukherjee and Friars (1970) reported that the realized h^2 of 12-week body weight ranged from 0.37 to 0.57 in different base populations. Johnson and Gowe (1962) observed that the growth curve of the turkey could be changed by selecting for increased body weight at different ages.

Little or no association between egg production and body weight was observed in earlier selection studies during the first few generations of selection for either increased egg production (Kosin and Becker, 1959; Shoffner and Leighton, 1962) or increased body weight (Ogasawara *et al.*, 1963; Mukherjee and Friars, 1970). Cook *et al.* (1962), Clayton (1971), and Arthur and Abplanalp (1975) estimate the genetic correlation between body weight and egg production was -0.1.

The purpose of the present study was to analyze direct and correlated responses to long-term selection for increased 16-week body weight of turkeys over 30 generations of selection and to estimate changes in genetic parameters. To do this, changes occurring at 10 generation intervals were analyzed.

Materials and Methods

The base population was a randombred control (RBC2) established in 1966 from pooled reciprocal crosses of two commercial large-bodied strains (Nestor *et al.*, 1969). The RBC2 line was maintained using a mating system (Nestor, 1977) utilizing 36 parental pairs. A subline (F) of the RBC2 line was initiated by mass selection for increased 16-week body weight. The F line was maintained with 36 parental pairs from Generations 1 through 21. In Generation 22 and later, the F line was reproduced with 36 males and 72 females (each male being mated with two females).

Offspring from the RBC2 and F lines were fed a declining protein six-ration system (Naber and Touchburn, 1970) during the growing period based on the schedule for males. Some minor improvements were made in the rations during the course of the selection study. However, in all generations, both lines were fed the same rations.

Body weights were recorded at 8, 16, and 20 (Generations 9 through 30) and 24 (Generations 1 through 8) weeks of age. At 16 weeks of age in some generations, shank width and length (Nestor *et al.*, 1985) were measured, and the birds were subjectively rated for walking ability. Each bird was given a rating of from 1 to 5, with 1 indicating that the bird had legs without any lateral deviation and had no difficulty walking, and 5 representing birds whose legs exhibited extreme lateral deviation or had extreme difficulty walking, or both. Ratings of 2, 3, and 4 represented intermediate values between these extremes.

Selected males of the RBC2 and F lines were housed in a curtain-sided pole shelter at 20 or 24 weeks of age in Generations 1 through 27 and in a windowless house at 20 weeks of age in Generations 28 through 30. Beginning at stimulatory lighting (14 hours of light per day), the breeder males were fed a ration containing 15.3% crude protein, 0.93% calcium, 0.62% phosphorus, and 2,963 kcal/kg metabolizable energy.

Selected females were housed in a windowless breeder house and exposed to simulated declining daylight conditions until eight weeks prior to stimulatory lighting. At this time, light was restricted to six hours per day. The hens were given stimulatory lighting of 14 hours light per day at an intensity of 51 lx when they were approximately 39 weeks of age. The hens were fed a ration containing 17.6% crude protein, 2.25% calcium, 0.64% available phosphorus, and 2,751 kcal/kg metabolizable energy beginning one week prior to stimulatory lighting.

Egg production, in general, was recorded for a 180-day egg production period beginning with the first egg laid. The egg records were analyzed to obtain days required from stimulatory lighting to production of the first egg and measurements of intensity of lay (rate of lay and maximum and average clutch length) and broodiness (total days lost from periods of nonproduction of five or more consecutive days) according to the methods presented by Nestor (1972).

Each hen was artificially inseminated twice on the week the first egg was laid and biweekly thereafter for the RBC2 line and for Generations 1 through 16 of the F line. After Generation 16, hens of the F line were inseminated weekly. Volume of semen inseminated per hen varied but was almost always greater than the minimum amount (0.025 cc) generally recommended for maximum fertility. Fertility and hatch of fertile eggs were recorded for a 12-week hatching period beginning when the

hens first attained an egg production level of approximately 50%. Body weights of the females were also obtained at this time and used as a measure of adult body weight. Egg weight was obtained by group weighing all eggs laid by each hen every two weeks throughout the 12-week hatching period.

Data Analysis

The average increase in inbreeding per generation was calculated from one-half the reciprocal of the effective population size (Falconer, 1964). The effective population size was based on variation in family size.

Changes in traits over generations were estimated by the linear and quadratic regression coefficients of line means on generations. The significance of the regression coefficients was evaluated by *t* tests. Values of the F line were expressed as a deviation from the RBC2 line. Whenever possible, the selection period was divided into 10 generation intervals to evaluate changes in genetic parameters associated with selection. A separate analysis was also done on the entire 30 generations. In order to evaluate the effect of selection over the entire period, a one-way ANOVA of traits was used to estimate the effect of line in the 30th generation of selection.

Intended and actual selection differentials of 16-week BW in the F line were obtained. The intended selection differential was defined as the average of the selected individuals minus the population mean, and the actual selection differential was the intended selection differential weighted for the number of offspring produced.

Realized h^2 was estimated by the linear regression of selection responses in the F line, corrected for environmental fluctuations by expressing the values as deviations from the RBC2 line, on accumulated actual selection differentials by generations. The standard errors of the regression coefficient served as an approximation of the standard errors of the h^2 estimates.

Results

The total increase in inbreeding over the 30 generations was 12.4 and 27.1%, respectively, in the RBC2 and F lines. The respective average increase per generation was 0.41 and 0.90%.

Direct and Correlated Changes in the F Line

The total changes in growth and reproduction traits observed in the F line are given in Tables 1 and 2, respectively. After 30 generations of selection, body weight of the F line approached twice that of its base population, the RBC2 line. This increase in body weight of the F line was associated with an increase in shank width and length and poorer walking ability (higher walking ability scores). Genetic increases in 16-week body weight in the F line had no influence on mortality to eight weeks of age but was associated with decreased total number of eggs laid primarily due to decreased intensity of lay as measured by clutch length and rate of lay. There was no significant change in broodiness in the F line. Days to first egg from stimulatory lighting and egg weight increased in the F line. Hatch of fertile eggs decreased, but there was no change in fertility in the F line.

Changes in growth traits in the F line as measured by linear regression coefficients of deviation from the RBC2 line on generations of selection are presented in Table 3. All of the changes in growth traits in the F line were linear because no significant quadratic regression coefficients were observed. Gains in 16-week body weight were greater in Generations 1 to 10 than in Generations 11 to 20. The largest gains in 16-week body weight were observed in Generations 21 to 30 when the number of breeders in the F line was increased. Changes in shank measurements were positive and linear over the period in which these traits were recorded.

Changes per generation in reproduction traits in the F line are presented in Table 4. The changes in egg production were not consistent over the entire selection period, and there was a significant negative quadratic regression coefficient for the entire period. The largest decrease in egg production occurred in the first 10 generations of selection in the F line. No significant change in egg production was observed in Generations 11 to 20, but there was a loss of one egg per hen per generation during Generations 21 to 30. The increase in days from stimulatory lighting to production of the first egg occurred only in the first 10 generations of selection in the F line. The largest decreases in intensity of lay (maximum clutch length, average clutch length, and rate of lay) and increases in egg weight

in the F line occurred during the first 10 generations of selection. There was no significant change in total days broody in the F line. The linear regression coefficient of hatch of fertile eggs on generations was negative for all periods of measurement but significant only for Generations 11 to 20 and 1 to 30. No linear changes were noted for fertility in the F line, but the quadratic regression coefficient was positive and significant for the entire period.

Selection Intensity, Selection Differentials, and Realized Heritabilities

The number of birds available at 16 weeks of age and percentage of offspring selected to reproduce the F line are shown in Table 5. For both sexes combined, the percentage of offspring selected in the F line was similar in Generations 1 to 10 and 21 to 30 even though population size of the F line was increased in the latter period. Selection intensity, as measured by percentage selected, was not as great (percentage selected was greater) in Generations 11 to 20.

Intended and actual selection differentials did not consistently differ in the F line (Table 5). Realized h^2 of 16-week body weight in the F line as estimated by the linear regression of response on accumulated actual selection differentials was higher in males than in females and declined with selection. For both sexes combined, the decline was about three or four percentage points for each 10 generations of selection. The decline in realized h^2 of 16-week body weight did not appear to be the same in the two sexes. For males, the h^2 declined about two percentage points in Generations 11 to 20 relative to that in Generations 1 to 10 and further declined by about six percentage points in Generations 21 to 30. A decline of about six percentage points in h^2 occurred in females from Generations 1 to 10 to Generations 11 to 20 with no further change in Generations 21 to 30.

Discussion

Long-term selection studies for increased body weight have been conducted at Virginia using chickens (Dunnington and Siegel, 1996), at Georgia (Anthony *et al.*, 1996; Marks, 1996) and in Ohio (Anthony *et al.*, 1996) using Japanese Quail, and in Ohio (Nestor *et al.*, 1996c) using turkeys. Direct responses to selection have been measured in all of the selection studies. In general, realized h^2 estimates declined with selection in chickens (Liu *et al.*, 1994), in Japanese quail in Georgia (Marks, 1996) and Ohio (Nestor *et al.*, 1996a), and in the present study with turkeys. However, the realized h^2 was still greater than 0.20 at the 30th generation of selection in the F line indicating that a plateau in response is not likely in the near future of this line.

Measurements of correlated response were made only periodically in the chicken selection study in Virginia and in the Japanese quail selection study in Georgia. In the Japanese quail selection study (Nestor *et al.*, 1996b) in Ohio and in the present study using turkeys, many correlated traits were measured each generation so that changes with selection in the correlated traits could be measured. The results of the present study indicate that the genetic relationship, particularly in magnitude, between 16-week body weight and many correlated traits changed with selection. The changes in number of eggs produced per hen was negative and significant in the F line only for Generations 1 to 10 and 21 to 30. For intensity of lay traits (maximum and average clutch length and rate of lay), the changes were larger in early generations of selection. The days required from stimulatory lighting to production of the first egg increased only in Generations 1 to 10 of the F line. A significant decline in hatch of fertile eggs in the F line was observed only in Generations 11 to 20. The results of the present study indicate that the magnitude of genetic correlations, as well as the h^2, may change with selection.

References

Abplanalp, H., F. X. Ogasawara, and V. S. Asmundson. 1963. Influence of selection for body weight at different ages on growth of turkeys. *Br. Poult. Sci.* 4:71-82.

Anthony, N. B., K. E. Nestor, and H. L. Marks. 1996. Short-term selection for four-week body weight in Japanese quail. *Poultry Sci.* 75:1192-1197.

Arthur, J. A. and H. Abplanalp. 1975. Linear estimates of heritability and genetic correlation for egg production, body weight, conformation and egg weight of turkeys. *Poultry Sci.* 54:11-23.

Clayton, G. A. 1971. Egg production in turkeys. *Br. Poult. Sci.* 12:463-474.

Cook, R. E., W. L. Blow, C. C. Cockerham, and

E. W. Glazener. 1962. Improvement of reproductive traits and body measurements of turkeys. *Poultry Sci.* 41:556-563.

Dunnington, E. A., and P. B. Siegel. 1996. Long-term divergent selection for eight-week body weight in White Plymouth Rock chickens. *Poultry Sci.* 75:1168-1179.

Falconer, D. S. 1964. Introduction to Quantitative Genetics. Oliver and Boyd, Edinburgh, U.K.

Havenstein, G. B., K. E. Nestor, V. D. Toelle, and W. L. Bacon. 1988. Estimates of genetic parameters in turkeys. 1. Body weight and skeletal characteristics. *Poultry Sci.* 67:1378-1387.

Johnson, A. S. and R. S. Gowe. 1962. Modification of the growth pattern of the domestic turkey by selection at two ages. Pages 57-63. *In*: Proceedings of the 12th World's Poultry Congress, Sydney, Australia.

Kosin, I. L. and W. A. Becker. 1959. Shifts in the phenotype of lines within a turkey strain, following several generations of single-trait selective breeding. *Poultry Sci.* 38:1220. (Abstr.)

Liu, G., E. A. Dunnington, and P. B. Siegel. 1994. Responses to long-term divergent selection for eight-week body weight in chickens. *Poultry Sci.* 73:1642-1650.

Marks, H. L. 1996. Long-term selection for body weight in Japanese quail under different environments. *Poultry Sci.* 75:1198-1203.

McCartney, M. G. 1961. Heritabilities and correlations for body weight and conformation in a randombred population of turkeys. *Poultry Sci.* 40:1694-1700.

McCartney, M. G., K. E. Nestor, and W. R. Harvey. 1968. Genetics of growth and reproduction in the turkey. 2. Selection for increased body weight and egg production. *Poultry Sci.* 47:981-990.

Mukherjee, T. K. and G. W. Friars. 1970. Heritability estimates and selection responses of some growth and reproductive traits in control and early growth of selected strains of turkeys. *Poultry Sci.* 49:1215-1222.

Naber, E. C. and S. P. Touchburn. 1970. *Ohio Poultry Rations.* Ohio Cooperative Extension Service Bulletin 343. The Ohio State University, Columbus, Ohio.

Nestor, K. E. 1972. Broodiness, intensity of lay, and total egg production of turkeys. *Poultry Sci.* 51:86-92.

Nestor, K. E. 1977. The use of a paired mating system for the maintenance of experimental populations of turkeys. *Poultry Sci.* 56:60-65.

Nestor, K. E., W. L. Bacon, Y. M. Saif, and P. A. Renner. 1985. The influence of genetic increases in shank width on body weight, walking ability, and reproduction of turkeys. *Poultry Sci.* 64:2248-2255.

Nestor, K. E., M. G. McCartney, and N. Bachev. 1969. Relative contribution of genetics and environment to turkey improvement. *Poultry Sci.* 48:1944-1949.

Nestor, K. E., M. G. McCartney, and W. R. Harvey. 1967. Genetics of growth and reproduction in the turkey. 1. Genetic and non-genetic variation in body weight and body measurements. *Poultry Sci.* 46:1374-1384.

Nestor, K. E., W. L. Bacon, N. B. Anthony, and D. O. Noble. 1996a. Divergent selection for body weight and yolk precursor in *Coturnix coturnix japonica*. 10. Response to selection over thirty generations. *Poultry Sci.* 75:303-310.

Nestor, K. E., W. L. Bacon, N. B. Anthony, and D. O. Noble. 1996b. Divergent selection for body weight and yolk precursor in *Coturnix coturnix japonica*. 11. Correlated responses over thirty generations. *Poultry Sci.* 75:472-477.

Nestor, K. E., D. O. Noble, J. Zhu, and Y. Moritsu. 1996c. Direct and correlated responses to long-term selection for increased body weight and egg production in turkeys. *Poultry Sci.* 75:1180-1191.

Ogasawara, F. X., H. Abplanalp, and V. S. Asmundson. 1963. Effect of selection for body weight on reproduction in turkey hens. *Poultry Sci.* 42:838-842.

Shoffner, R. N. and A. T. Leighton. 1962. Photoperiodicity and selection for egg production in the turkey (*Meleagris gallapavo*). Pages 37-42. *In*: Proceedings of the 12th World's Poultry Congress, Sydney, Australia.

Table 1. Effect of Selecting Turkeys Over Thirty Generations for Increased 16-Week Body Weight on Body Weight at Various Ages, Shank Measurements, Walking Ability, and Mortality.

	Line[1]		
Variable	RBC2	F	F-RBC2
Male body weight, kg			
8 wk	2.60	4.59	1.99***
16 wk	7.66	13.84	5.82***
20 wk	9.80	17.86	8.06***
Female body weight, kg			
8 wk	2.07	4.01	1.94***
16 wk	5.79	10.63	4.84***
20 wk	6.44	12.29	5.85***
Adult[2]	9.48	17.37	7.89***
Shank length, cm			
Males	19.95	22.12	3.27***
Females	16.54	18.10	1.56***
Shank width, mm			
Males	13.44	16.71	3.27***
Females	11.63	15.48	3.85***
Walking ability score[3]			
Males	1.54	3.04	1.50***
Females	1.38	2.89	1.51***
Mortality, 0 to 8 wk, %	7.0	11.1	4.1

[1] RBC2 = randombred control line; F = subline of RBC2 selected for increased 16-week BW.

[2] Body weight when hens first achieved 50% egg production.

[3] Birds were subjectively rated at 16 weeks of age from 1 to 5, with 1 representing birds whose legs did not have any defects and had no difficulty walking, and 5 indicating birds whose legs exhibited extreme lateral deviation or had great difficulty walking. Ratings of 2, 3, and 4 represented intermediate values.

*** $P \leq 0.001$.

Table 2. Effect of Selecting Turkeys Over Thirty Generations for Increased 16-Week Body Weight on Reproduction Traits.

	Line[1]		
Variable	RBC2	F	F-RBC2
Egg production[2], no./hen	92.6	68.7	-23.9***
Days to first egg[3]	20.1	24.4	4.3***
Clutch length[2], d			
Maximum	6.8	3.4	-3.4***
Average	2.20	1.46	-0.74***
Total days broody[2,4]	28.1	35.2	7.1
Rate of lay[2,5], %	60.8	47.0	-13.8***
Egg weight[6], g	89	98	9***
Fertility[6], %	89	87	-2
Hatch of fertile eggs[6], %	85	75	-10***

[1] RBC2 = randombred control line; F = subline of RBC2 selected for increased 16-week BW.

[2] Based on an 180-day production period.

[3] Days from beginning of stimulatory lighting (14 hours/day) to production of first egg.

[4] Total number of days lost during pauses in egg production of five or more consecutive days.

[5] Rate of lay = number of eggs laid. (180 d-total days broody).

[6] Based on the first 12-weeks of egg production.

*** $P \leq 0.001$.

Table 3. Linear Regression Coefficients of Growth Traits on Generations of Selection in the F Line After Adjustment for Changes in the Environment.[1]

	Generations of Selection			
Variable	1 to 10	11 to 20	21 to 30	0 to 30
Male body weights, kg				
8 wk	0.049***	0.054***	0.072**	0.072***
16 wk	0.193***	0.115***	0.258***	0.185***
20 wk	0.193***	0.347***	0.535***, [2]	
Female body weights, kg				
8 wk	0.049***	0.050***	0.094***	0.066***
16 wk	0.160***	0.115***	0.213***	0.152***
20 wk	0.158***	0.245***	0.185***, [2]	
Adult[3]	0.226***	0.260**	0.332***	0.225***
Shank length, cm				
Males				0.048***, [4]
Females				0.046***, [4]
Shank width, mm				
Males				0.121***, [4]
Females				0.140***, [4]
Walking ability score[5]				
Males				0.045***, [4]
Females				0.116***, [6]
Mortality, 0 to 8 wk, %	-0.250	-0.100	0.439	-0.043

[1] The F line was selected for increased 16-week BW. In order to remove environmental variation, values for the randombred control were subtracted from those of the F line prior to regression analysis.

[2] Based on Generations 9 through 30.

[3] Body weight when hens first achieved approximately 50% egg production.

[4] Based on Generations 14 through 30.

[5] Birds were subjectively rated from 1 to 5, with 1 representing birds whose legs did not have any defects and had no difficulty walking, and 5 indicating birds whose legs exhibited extreme lateral deviation or great difficulty walking. Ratings of 2, 3, and 4 represented intermediate values.

[6] Based on Generations 21 through 30.

*** $P \leq 0.001$.

Table 4. Linear Regression Coefficients of Reproduction Traits on Generations of Selection in the F Line After Adjustment for Changes in the Environment.[1]

	Generations of Selection			
Variable	1 to 10	11 to 20	21 to 30	1 to 30
Egg production[2], no./hen	-2.961**	-0.113	-1.061*	-0.231[3]
Days to first egg[4]	0.143**	0.251	0.032	0.115[3]
Clutch length[2], d				
Maximum	-0.364**	-0.092	-0.157*	-0.103***
Average	-0.068**	-0.050*	-0.021	-0.026***
Total days broody[2,5]	2.405	-0.261	0.699	-0.418
Rate of lay[2,6], %	-0.011***	-0.010*	-0.003	-0.004**
Egg weight[7], g	0.224*	-0.194	0.181	0.311***
Fertility[7], %	-1.194	0.384	0.667	-0.011[8]
Hatch of fertile eggs[7], %	-0.133	-0.827*	-0.067	-0.276***

[1] The F line was selected for increased 16-week BW. In order to remove environmental variation, values for the randombred control were subtracted from those of the F line prior to regression analysis.

[2] Based on a 180-day production period.

[3] Significant ($P \leq 0.05$) negative quadratic regression coefficient.

[4] Days from beginning of stimulatory lighting (14 hours/day) to production of first egg.

[5] Total number of days lost during pauses in egg production of five or more consecutive days.

[6] Rate of lay = number of eggs laid. (180d-total days broody).

[7] Based on a 12-week period.

[8] Significant ($P \leq 0.05$) positive quadratic regression coefficient.

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

Table 5. Selection Intensity, Selection Differentials, and Realized Heritabilities in the F Line.[1]

Variable	Generations of Selection			
	1 to 10	11 to 20	21 to 30	1 to 30
Number of birds[2]				
RBC2 line	376	364	324	355
F line	305	275	384	322
Percentage selected				
Males	24.8	32.4	18.0	25.1
Females	23.2	29.8	31.1	28.0
Both sexes	24.0	31.1	24.6	26.5
Selection differentials, kg[3]				
Males				
I	0.6269	0.6201	1.3054	0.8508
A	0.6178	0.6128	1.3090	0.8465
I-A	-0.0091	-0.0073	0.0036	-0.0043
Females				
I	0.5372	0.4409	0.6087	0.5289
A	0.5398	0.4373	0.6141	0.5304
I-A	0.0026	-0.0036	0.0054	0.0015
Realized heritability				
Linear regression[4]				
Males	0.337±0.030	0.320±0.041	0.320±0.026	0.285±0.006
Females	0.280±0.021	0.217±0.036	0.218±0.028	0.234±0.006
Both sexes	0.309±0.022	0.268±0.033	0.242±0.026	0.254±0.007
Final generation				
Males	0.392	0.341	0.306	0.306
Females	0.321	0.255	0.241	0.241
Both sexes	0.356	0.298	0.274	0.274

[1] The F line was developed from a randombred control population (RBC2) by selecting for increased 16-week body weight.

[2] Based on birds alive at 16 weeks of age.

[3] I = intended selection differential (mean of selected birds minus mean of all birds); A = intended selection differential weighted by the number of offspring hatched.

[4] Heritibility ± standard error.

The Effect of Selection for Increased Body Weight, Egg Production, and Shank Width on Developmental Stability in Turkeys

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Abstract

Bilateral asymmetry is a measure of developmental stability. At 20 weeks of age, the right and left shank length, shank width (width laterally at the dew claw), shank depth (width perpendicular to the dew claw), and face length (between auditory canal opening and the posterior junction of the upper and lower mandible) were measured in three randombred control and three selected lines of turkeys. The lines were grown intermingled, with the sexes being grown in different houses. The selected lines had been selected for increased egg production (38 generations), increased 16-week body weight (32 generations), or increased shank width (19 generations), and had a higher level of inbreeding (average = 36.9%) than the randombred controls (average = 11.6%). The bilateral differences (right minus left) were analyzed for the presence of asymmetry. In order to adjust for possible scaling effects, relative asymmetry (RA), in which the mean of the absolute differences between sides was divided by the mean of the two sides and the resulting value multiplied by 100, was used as a measure of bilateral asymmetry. The randombred control and selected lines were contrasted to study the effect of homozygosity on RA. Likewise, the large-bodied lines (F, FL, and RBC3) were contrasted to the small-bodied lines (RBC1, E, and RBC2) to study the effect of body weight on RA.

The level of asymmetry for the traits was ranked: face length > shank width = shank depth > shank length. The individual lines differed in RA for shank length and shank width for both sexes and for shank depth and face length in females. In general, the influence of body weight, as measured in the contrast of large-bodied and small-bodied lines, on RA was larger than that of homozygosity, as measured by the contrast of the selected and randombred control lines.

Introduction

Developmental homeostasis may be reduced by genetic selection within populations (Lerner, 1954). Individual animals have multiple copies of morphological structures, and if development of such structures on the two sides of a bilaterally symmetrical animal are under genetic control, then both sides are expected to be identical because they are products of the same genome (Leary and Allendorf, 1989). There are three possible types of asymmetry — fluctuating (FA), directional (DA), and antisymmetry (AS) (Van Valen, 1962). Fluctuating asymmetry is defined as the differences of the right and left sides having a mean of zero with normal variation. For DA, the differences are not zero but the variation is normal. If the differences between sides have a mean of zero with non-normal distribution (usually bimodal), the differences are AS.

Fluctuating asymmetry is generally thought to be a good measure of environmental and genetic

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(inbreeding and founder effects) stresses in laboratory and natural populations (Van Valen, 1962). However, DA and AS are not as useful for measurement of developmental stability, since these forms of asymmetry might be controlled genetically (Palmer and Strobeck, 1992).

Generally, FA and homozygosity of genes are positively correlated (Leary and Allendorf, 1989; Parsons, 1990), but exceptions do occur (Thoday, 1958; Palmer and Strobeck, 1986). A positive correlation was observed between the coefficient of variation of a trait and level of FA (Clarke, 1998).

Bilateral asymmetry has been studied in commercial (Møller *et al.*, 1995) and experimental (Yang *et al.*, 1997, 1998; Yang and Siegel, 1998) populations of chickens. Møller *et al.* (1995) reported that FA was greater in fast-growing breeds than in slower-growing ones. When fast-growing chickens were kept at a density of 20, 24, or 28 chickens per square meter, FA was positively associated with population density. Yang *et al.* (1997) studied the relative asymmetry [RA; ((*left side-right side*)) (left side + right side) 2)) X 100)] of shank length, shank width (perpendicular to the spur), face length (distance between the auditory canal and posterior junction of the upper and lower mandibles), and length and weight of the first primary flight feather in lines of chickens selected 23 generations for high or low antibody response to sheep red blood cells, sublines of the high and low lines that had been relaxed selected for eight generations, and reciprocal F_1 crosses between the selected lines. Rankings among the various genetic stocks for RA, based on the means for the five traits, showed that the selected lines exhibited greater RA than the crosses between them. The RA of the two lines in which selection had been relaxed was similar to the RA of the selected lines. In another study, Yang and Siegel (1998) studied asymmetry in the length and weight of the shank, the length and weight of the first primary flight feather, length of the ceca, and weight of the lungs in the high and low antibody response lines and reciprocal F_1 crosses among them. All forms of asymmetry were observed in the various subgroups (20 FA, 12 AS, and 8 DA). The heterosis of RA in the crosses was negative for all traits and significant for shank length, length and weight of the first primary feather, and ceca lengths in some groups.

Yang *et al.* (1998) studied the developmental stability at 240 days of age in a line of chickens selected 39 generations for low 56-day BW and a subline of the low line that had been maintained with relaxed selection. The selected line was divided into layers and non-layers. Relative asymmetry was similar among the three groups for shank length, shank depth, and face length. Values for first primary feather weight and length were higher for both low-weight selected groups than the relaxed selected subline. The overall mean of asymmetries was higher for both selected groups than the relaxed selected group.

The results of Yang *et al.* (1997, 1998) and Yang and Siegel (1998) suggest that asymmetry in bilateral traits may be a good measure of genetic stress and developmental stability. Turkeys have been selected long-term for increased body weight, increased egg production, and increased shank width. The purpose of the present study was to study developmental stability in the selected lines and three randombred control lines by measuring asymmetry of bilateral traits.

Materials and Methods

Lines of Turkeys

A line (E) of turkeys was selected long-term (38 generations) for increased egg production for various periods of production (McCartney *et al.*, 1968; Nestor *et al.*, 1996). The E line was started from a randombred control population (RBC1; McCartney, 1964) and selection was based on the offspring from the best dams. A line (F) mass selected long-term (32 generations) for increased 16-week body weight (Nestor, 1977; Nestor *et al.*, 1996) was developed from a different randombred control population (RBC2; Nestor *et al.*, 1969). A subline (FL; Nestor *et al.*, 1985) of the F line was initiated by mass selecting only for increased shank width with 19 generations of selection completed at the time of the present study. A third randombred control line (RBC3; Noble *et al.*, 1995) was developed from reciprocal crosses of the F line and a commercial sire line. The RBC3 line had been maintained for 12 generations.

The randombred control lines were maintained without conscious selection by a paired mating system using 36 parental pairs. The number of parental pairs used to reproduce the E line varied from 36 to 72 over the course of selection. For the F line, 36 parental pairs were used to reproduce the line through Generation 21, after which

time 36 males were mated to 72 females with two females being assigned to each male. The FL line was maintained with 36 parental pairs from Generation 1 through 12; thereafter, the line was maintained by mating 36 males to 54 females.

Management and Measurement of Birds

The birds were produced in a single hatch based on a two-week collection of eggs from parents of similar age. The birds were grown, sexes separate, in confinement in separate houses. All birds were provided with a declining protein five-ration system (Naber and Touchburn, 1970) based on the schedule for males. Continuous lighting was provided from hatching to eight weeks of age at which time the length of the light day was reduced to 12 hours. At 16 weeks of age, the amount of light per day was reduced to 10 hours and remained at this level.

Body weight was recorded at 8, 16, and 20 weeks of age. At 20 weeks of age, measurements of shank length, shank width (laterally at the dew claw), shank depth (perpendicular to the dew claw), and face length (between auditory canal opening and the posterior junction of the upper and lower mandible) were made to the nearest tenth of a unit on both sides of the body by the same person. The number of turkeys in each line and sex subgroup was 50 or 51.

Estimation of Inbreeding

The average increase in inbreeding per generation was calculated from one-half the reciprocal of the effective population size. Effective population size was based on the total number of parents and variation in family size among the parents. The amount of inbreeding in the F line at the time of formation of the FL was added to the values for the FL line.

Statistical Analysis

The sexes were analyzed separately. Data on asymmetry were expressed for the right side minus the left side as signed and absolute values. The RA was obtained by dividing the absolute differences between sides by the average value of both sides and multiplying by 100. The signed bilateral asymmetry values were tested for normality with mean zero by the Shapiro-Wilk statistic and one sample t test (SAS Institute, 1988). Traits within line and sex subgroups were classified into type of asymmetry (FA, DA, or AS) based on the mean and normality of variation of the signed differences (Van Valen, 1962).

The influence of line on the various traits was tested by a one-way ANOVA. Means were separated by repeated t tests. In order to study the effect of body weight, contrasts were made of the small-bodied (RBC1, E, and RBC2) and large-bodied (F, FL, and RBC3) lines. Contrasts were also used to compare randombred (RBC1, RBC2, and RBC3) and selected (E, F, and FL) lines.

Results

The total accumulated inbreeding was 16.4, 13.4, and 5.1%, respectively, for the RBC1, RBC2, and RBC3 lines. The respective average increase in inbreeding per generation was 0.43, 0.42, and 0.43%. For the selected lines, the total accumulated inbreeding was 52.4, 28.1, and 30.2%, respectively, in the E, F, and FL lines. The increase in inbreeding per generation was higher in the E line (1.38%) than in the F (0.88%) and FL (0.95%) lines. Thus, the selected lines not only had higher accumulated inbreeding but a faster rate of inbreeding than the randombred control lines.

All individual turkey lines were significantly different in body weight at 8, 16, and 20 weeks of age within sexes (Table 1). Selection for increased egg production decreased body weight, whereas body weight was increased in the F and FL lines relative to the RBC2 line. The body weight of the randombred control lines was ranked in the order: RBC3 > RBC2 > RBC1. In general, the large-bodied lines were more than twice as heavy as the small-bodied lines.

With the exception of the FL line, line differences in average values for bilateral measurements of shank length, shank width, shank depth, and face length generally were similar to those in body weight (Table 2). The FL line had greater shank width (the selected trait) and shank depth than the F line even though body weight of the F line was more than 25% larger than the body weight of the FL line (Table 1). The RBC3 line had greater body weight than the FL line (Table 1), but the shank measurements were greater in the FL line than in the RBC3 line, and face length was similar in the FL and RBC3 lines (Table 2).

The signed and absolute differences and RA between the right and left shank lengths differed significantly among individual turkey lines for

Table 1. Body Weights of Individual Turkey Lines and Groups of Lines.

Lines[1]	Males			Females		
	8 wk	16 wk	20wk	8 wk	16 wk	20 wk
	(kg)					
RBC1	2.012[e]	6.030[e]	7.695[e]	1.814[e]	4.329[e]	5.310[e]
E	1.480[f]	4.950[f]	6.354[f]	1.346[f]	3.573[f]	4.460[f]
RBC2	2.614[d]	7.695[d]	9.765[d]	2.259[d]	5.355[d]	6.615[d]
F	5.130[a]	14.310[a]	17.685[a]	4.437[a]	10.755[a]	12.870[a]
FL	3.906[c]	11.205[c]	13.725[c]	3.359[c]	8.055[c]	9.675[c]
RBC3	4.100[b]	11.655[b]	14.670[b]	3.609[b]	8.505[b]	10.305[b]
Randombred	2.909[y]	8.460[y]	10.710[y]	2.561[y]	6.063[y]	7.410[y]
Selected	3.505[x]	10.155[x]	12.588[x]	3.047[x]	7.461[x]	9.002[x]
Small-bodied	2.035[y]	6.225[y]	7.935[y]	1.806[y]	4.419[y]	5.462[y]
Large-bodied	4.379[x]	12.390[x]	15.360[x]	3.802[x]	9.105[x]	10.950[x]

[a-f] Individual line means within columns with no common superscript are significantly different ($P \leq 0.05$).

[x-y] Means within groups of lines in columns with no common superscript are significantly different ($P \leq 0.05$).

[1] RBC1, RBC2, RBC3 = randombred control lines; E = subline of RBC1 selected for increased egg production; F = subline of RBC2 selected for increased 16-week BW; FL = subline of F selected for increased shank width; small-bodied = RBC1, E, and RBC2 lines; and large-bodied = F, FL, and RBC3 lines.

Table 2. Average Shank Length, Shank Width,[1] Shank Depth,[2] and Face Length[3] at 20 Weeks of Age in Individual Turkey Lines and Groups of Lines.

Lines[4]	Males				Females			
	Shank Length (cm)	Shank Width	Shank Depth	Face Length	Shank Length (cm)	Shank Width	Shank Depth	Face Length
		(mm)				(mm)		
RBC1	19.54[e]	12.90[e]	19.64[e]	38.27[c]	15.48[e]	11.51[d]	17.29[e]	33.01[c]
E	19.04[f]	12.28[f]	19.10[f]	35.57[d]	15.15[f]	10.84[e]	16.41[f]	31.81[d]
RBC2	20.08[d]	13.84[d]	21.65[d]	38.63[c]	16.20[d]	11.86[d]	18.51[d]	33.42[c]
F	22.02[a]	18.37[b]	27.14[b]	42.96[a]	17.84[a]	18.26[b]	23.52[b]	36.78[a]
FL	21.42[b]	23.95[a]	28.08[a]	41.74[b]	17.21[b]	21.39[a]	24.09[a]	35.41[b]
RBC3	20.99[c]	17.14[c]	25.04[c]	41.10[b]	16.88[c]	16.13[c]	21.68[c]	35.61[b]
Randombred	20.20[y]	14.62[y]	22.11[y]	39.33[y]	16.19[y]	13.17[y]	19.16[y]	34.01[y]
Selected	20.83[x]	18.20[x]	24.77[x]	40.09[x]	16.73[x]	16.83[x]	21.34[x]	34.67[x]
Small-bodied	19.55[y]	13.01[y]	20.13[y]	37.49[y]	15.61[y]	11.40[y]	17.40[y]	32.75[y]
Large-bodied	21.48[x]	19.82[x]	26.75[x]	41.93[x]	17.31[x]	18.59[x]	23.10[x]	35.93[x]

[a-f] Individual line means within columns with no common superscript are significantly different ($P \leq 0.05$).

[x-y] Means within groups of lines in columns with no common superscript are significantly different ($P \leq 0.05$).

[1] Lateral width at the dew claw.

[2] Width perpendicular to the dew claw.

[3] Distance between auditory canal opening and the posterior junction of the upper and lower mandible.

[4] RBC1, RBC2, RBC3 = randombred control lines; E = subline of RBC1 selected for increased egg production; F = subline of RBC2 selected for increased 16-week BW; FL = subline of F selected for increased shank width; small-bodied = RBC1, E, and RBC2 lines; and large-bodied = F, FL, and RBC3 lines.

both males and females and were highest in the FL line (Table 3). The RA ranged from 0.61 to 1.30% in males and from 0.71 to 1.65% in females for the various lines. Fluctuating asymmetry was observed for shank length in all line and sex subgroups except for FL males and RBC1 females in which DA was found. When randombred and selected lines were compared as a group, only the absolute difference in male shank length was significantly different with the selected lines exhibiting a larger difference. The signed and absolute differences and RA were larger in the large-bodied lines than in the small-bodied lines for both sexes.

The FL line also had the greatest RA for male (range = 1.53 to 3.37%) and female (range = 1.67 to 5.02%) shank width (Table 4), and line differences were evident for signed and absolute differences between sides of the body and RA in both sexes. In the comparison of the randombred and selected lines, only the absolute differences between sides were significantly higher for the selected lines in males while in females the selected lines had larger signed and absolute differences and RA than the randombred lines. The large-bodied lines had greater signed and absolute differences and RA than the small-bodied lines in both males and females. Fluctuating asymmetry was observed in all line and sex subgroups except for the RBC1 males for which DA was observed.

Even though shank depth and shank width were measured at the same location (at the dew claw) but in a different direction (anterior-posterior for depth and laterally for width), the results were quite different between the two measurements. For males, the RBC1 line had a significantly lower signed difference between sides for shank depth than the other lines, but no line difference in absolute difference or RA was observed. Males of the selected lines had a greater signed difference than the randombred control lines. Males of the large-bodied lines had greater signed and absolute differences between sides than the small-bodied lines,

Table 3. Lateral Asymmetry[1] in Shank Length in Individual Turkey Lines and Groups of Lines.

	Males				Females			
Lines[2]	Signed	Absolute	Relative[3]	Type[4]	Signed	Absolute	Relative[3]	Type[4]
	———(cm)———		—(%)—		———(cm)———		—(%)—	
RBC1	0.043^{abc}	0.176^{cd}	0.91^{bcd}	FA	0.074^{ab}	0.172^{bc}	1.11^{bc}	DA
E	-0.100^{bc}	0.130^{d}	0.68^{cd}	FA	0.016^{b}	0.108^{c}	0.71^{c}	FA
RBC2	0.020^{bc}	0.122^{d}	0.61^{d}	FA	0.020^{b}	0.141^{bc}	0.87^{bc}	FA
F	0.052^{ab}	0.259^{ab}	1.21^{ab}	FA	0.086^{ab}	0.204^{b}	1.14^{b}	FA
FL	0.136^{a}	0.280^{a}	1.30^{a}	DA	0.164^{a}	0.284^{a}	1.65^{a}	FA
RBC3	0.100^{ab}	0.212^{bc}	1.01^{abc}	FA	0.122^{a}	0.198^{b}	1.18^{b}	FA
Randombred	0.054	0.170^{y}	0.84		0.072	0.171	1.05	
Selected	0.059	0.223^{x}	1.06		0.089	0.198	1.17	
Small-bodied	0.018^{y}	0.143^{y}	0.73^{y}		0.037^{y}	0.140^{y}	0.90^{y}	
Large-bodied	0.096^{x}	0.250^{x}	1.17^{x}		0.124^{x}	0.229^{x}	1.32^{x}	

[a-d] Individual line means within columns with no common superscript are significantly different ($P \leq 0.05$).

[x-y] Means within groups of lines in columns with no common superscript are significantly different ($P \leq 0.05$).

[1] Right minus left.

[2] RBC1, RBC2, RBC3 = randombred control lines; E = subline of RBC1 selected for increased egg production; F = subline of RBC2 selected for increased 16-week BW; FL = subline of F selected for increased shank width; small-bodied = RBC1, E, and RBC2 lines; and large-bodied = F, FL, and RBC3 lines.

[3] ((| Right-left |) ÷ ((right + left) ÷ 2)) X 100.

[4] FA = fluctuating asymmetry (signed difference zero with normal variation); DA = directional asymmetry (signed difference not zero with normal variation).

Table 4. Lateral Asymmetry[1] in Shank Width[2] in Individual Turkey Lines and Groups of Lines.

Lines[3]	Males				Females			
	Signed	Absolute	Relative[4]	Type[5]	Signed	Absolute	Relative[4]	Type[5]
	——(mm)——		—(%)—		——(mm)——		—(%)—	
RBC1	-0.041[a]	0.269[c]	2.08[c]	DA	-0.028[a]	0.224[c]	1.94[b]	FA
E	-0.004[a]	0.188[c]	1.53[c]	FA	-0.026[a]	0.182[c]	1.67[b]	FA
RBC2	-0.078[a]	0.306[c]	2.17[bc]	FA	-0.063[a]	0.204[c]	1.71[b]	FA
F	-0.364[b]	0.582[b]	3.19[a]	FA	-0.556[b]	0.847[b]	4.61[a]	FA
FL	-0.180[ab]	0.800[a]	3.37[a]	FA	-0.152[a]	1.076[a]	5.02[a]	FA
RBC3	-0.188[ab]	0.520[b]	3.05[ab]	FA	-0.106[a]	0.714[b]	4.39[a]	FA
Randombred	-0.102	0.365[y]	2.43		-0.066[y]	0.381[y]	2.68[y]	
Selected	-0.183	0.523[x]	2.70		-0.245[x]	0.702[x]	3.77[x]	
Small-bodied	-0.041[y]	0.254[y]	1.93[y]		-0.039[y]	0.203[y]	1.77[y]	
Large-bodied	-0.244[x]	0.634[x]	3.20[x]		-0.271[x]	0.879[x]	4.67[x]	

[a-c] Individual line means within columns with no common superscript are significantly different ($P \leq 0.05$).

[x-y] Means within groups of lines in columns with no common superscript are significantly different ($P \leq 0.05$).

[1] Right minus left.

[2] Width measured laterally at the dew claw.

[3] RBC1, RBC2, RBC3 = randombred control lines; E = subline of RBC1 selected for increased egg production; F = subline of RBC2 selected for increased 16-week BW; FL = subline of F selected for increased shank width; small-bodied = RBC1, E, and RBC2 lines; and large-bodied = F, FL, and RBC3 lines.

[4] ((| Right-left |) ÷ ((right + left) ÷ 2)) X 100.

[5] FA = fluctuating asymmetry (signed difference zero with normal variation); DA = directional asymmetry (signed difference not zero with normal variation).

but there was no difference in RA. For males, the asymmetry was FA in three lines and DA in the other three lines. For females, line differences were evident in all three measurements. The RA ranged from 2.21 to 3.91%. No significant differences were observed between the randombred and selected lines. The large-bodied lines had greater signed and absolute differences and RA than the small-bodied lines. All lines exhibited FA except for the F line which exhibited DA.

The line comparisons were different for males and females for lateral asymmetry in face length (Table 6). A line difference was observed for the signed differences between sides in males but there was no significant line variation in absolute differences between sides or in RA. Lines differed for all three measures in females. There was no significant difference between the randombred control and selected lines or between the small-bodied and large-bodied lines in any measure for either males or females. Directional asymmetry was noted in E line males and females and in RBC2 females and the remaining line and sex subgroups exhibited FA.

No individual line difference was evident in the total or average RA of shank length, shank width, shank depth, and face length for males (Table 7). Likewise, there was no difference in total or average RA for males between the randombred control and selected lines and small-bodied and large-bodied lines. The total RA for all traits and average RA were 12.4 and 3.1%, respectively. Lines differed in total and average RA for females. The large-bodied lines had greater values than the small-bodied lines. There was no difference between the randombred and selected lines. The total and average RA for females was 12.5 and 3.1%, respectively.

The RA was largest for face length and least for shank length in both sexes (Table 8). Shank width and shank depth had RA values in males and females intermediate between face length and shank

Table 5. Lateral Asymmetry[1] in Shank Depth[2] in Individual Turkey Lines and Groups of Lines.

Lines[3]	Males				Females			
	Signed	Absolute	Relative[4]	Type[5]	Signed	Absolute	Relative[4]	Type[5]
	———(mm)———		—(%)—		———(mm)———		—(%)—	
RBC1	-0.118[b]	0.604	3.22	FA	0.039[c]	0.412[b]	2.38[b]	FA
E	0.423[a]	0.592	3.11	DA	0.076[c]	0.364[b]	2.20[b]	FA
RBC2	0.433[a]	0.578	2.67	DA	0.228[bc]	0.467[b]	2.52[b]	FA
F	0.535[a]	0.788	2.90	FA	0.492[ab]	0.925[a]	3.91[a]	DA
FL	0.540[a]	0.724	2.57	FA	0.208[bc]	0.744[a]	3.09[a]	FA
RBC3	0.254[a]	0.714	2.86	DA	0.556[a]	0.800[a]	3.67[a]	FA
Randombred	0.190[y]	0.632	2.92		0.274	0.560	2.86	
Selected	0.502[x]	0.701	2.86		0.259	0.678	3.07	
Small-bodied	0.249[y]	0.591[y]	3.00		0.114[y]	0.414[y]	2.37[y]	
Large-bodied	0.443[x]	0.742[x]	2.78		0.419[x]	0.823[x]	3.56[x]	

[a-c] Individual line means within columns with no common superscript are significantly different ($P \leq 0.05$).

[x-y] Means within groups of lines in columns with no common superscript are significantly different ($P \leq 0.05$).

[1] Right minus left.

[2] Width measured perpendicular at the dew claw.

[3] RBC1, RBC2, RBC3 = randombred control lines; E = subline of RBC1 selected for increased egg production; F = subline of RBC2 selected for increased 16-week BW; FL = subline of F selected for increased shank width; small-bodied = RBC1, E, and RBC2 lines; and large-bodied = F, FL, and RBC3 lines.

[4] ((| Right-left |) ÷ ((right + left) ÷ 2)) X 100.

[5] FA = fluctuating asymmetry (signed difference zero with normal variation); DA = directional asymmetry (signed difference not zero with normal variation).

length but significantly different from both. Shank width and shank depth did not differ in RA.

Discussion

Line differences in body weight, shank measurements, and face length were similar in all comparisons except those involving the FL line. Body weight and body measurements were positively correlated genetically in several lines (Johnson and Asmundson, 1957; Nestor *et al.*, 1967; Havenstein *et al.*, 1988). Selection for increased shank width in the FL line increased body weight and shank length (Nestor *et al.*, 1985), shank depth (unpublished data), and the relative amount of leg bones (Nestor *et al.*, 1988). The shank of the FL line is becoming more round as measured by the ratio of shank width to shank depth (unpublished data). Face length of the FL line was greater than expected based on BW in the present experiment so perhaps selection in the FL line is increasing the relative amount of bone throughout the body. Previous results have shown that selection for increased egg production in the E line was associated with decreases in body weight and in shank length (Nestor, 1971). Changes in BW and shank measurements in the F line have been previously reported (Nestor *et al.*, 1985).

The large line differences in BW and body measurements may have resulted in scaling effects (Palmer and Strobeck, 1986) in the signed and absolute differences between the two sides of the body. Therefore, to remove possible scaling effects, asymmetry was expressed as RA that adjusts for the trait mean (Thoday, 1958).

Selection and the resulting inbreeding increase homozygosity of genes. The comparison of the selected and randombred control lines in the present experiment should provide a reasonable model for studying the influence of homozygosity on bilateral asymmetry in turkeys. Fluctuating asymmetry and homozygosity of genes are positively correlated in natural populations and some labora-

Table 6. Lateral Asymmetry[1] in Face Length[2] in Individual Turkey Lines and Groups of Lines.

Lines[3]	Males				Females			
	Signed	Absolute	Relative[4]	Type[5]	Signed	Absolute	Relative[4]	Type[5]
	———(mm)———		—(%)—		———(mm)———		—(%)—	
RBC1	1.63^{ab}	2.27	5.85	FA	1.61^{a}	1.93^{ab}	5.88^{a}	FA
E	1.83^{a}	2.13	5.97	DA	1.19^{abc}	1.59^{ab}	5.01^{ab}	DA
RBC2	0.72^{b}	2.62	7.49	FA	0.90^{bc}	1.48^{b}	4.41^{b}	DA
F	1.45^{ab}	2.13	4.94	FA	1.46^{ab}	1.92^{ab}	5.21^{ab}	FA
FL	1.89^{a}	2.49	5.94	FA	1.48^{ab}	1.97^{a}	5.53^{ab}	FA
RBC3	1.51^{ab}	2.48	6.05	FA	0.64^{c}	1.96^{a}	5.47^{ab}	FA
Randombred	1.29	2.47	6.46		1.05	1.79	5.25	
Selected	1.72	2.37	5.61		1.38	1.83	5.25	
Small-bodied	1.39	2.34	6.43		1.23	1.67	5.10	
Large-bodied	1.62	2.25	5.64		1.19	1.95	5.40	

[a-c] Individual line means within columns with no common superscript are significantly different ($P \leq 0.05$).

[1] Right minus left.

[2] Distance between auditory canal opening and the posterior junction of the upper and lower mandible.

[3] RBC1, RBC2, RBC3 = randombred control lines; E = subline of RBC1 selected for increased egg production; F = subline of RBC2 selected for increased 16-week BW; FL = subline of F selected for increased shank width; small-bodied = RBC1, E, and RBC2 lines; and large-bodied = F, FL, and RBC3 lines.

[4] ((| Right-left |) ÷ ((right + left) ÷ 2)) X 100.

[5] FA = fluctuating asymmetry (signed difference zero with normal variation); DA=directional asymmetry (signed difference not zero with normal variation).

tory populations (Leary and Allendorf, 1989; Parsons, 1990; Palmer, 1996) but exceptions do occur (Thoday, 1958; Palmer and Strobeck, 1986). In chickens, Yang *et al.* (1997) and Yang and Siegel (1998) observed that, based on mean RA for five traits, lines selected for high or low antibody response to SRBC had greater RA than crosses among them. In the comparison of the selected and randombred control lines in the present study, RA was not different for shank length, shank depth, and face length in males and females, and shank width in males. For shank width in females, RA was larger in the selected lines than in the randombred control lines. When the total or average RA for all four traits was considered, there was no significant difference between the randombred control and selected lines. Among the individual selected lines, the E line had been selected for the longest period of time and had the highest level of inbreeding but exhibited among the lowest RA values for some traits (shank length, shank width, and female shank depth). Egg production is a component of fitness and the effect of the increased egg production in the E line could have masked the effect of homozygosity on developmental stability. However, the RBC3 line had the least inbreeding among the randombred control lines and had higher RA values for some traits than the other two randombred lines. Thus, it appears that homozygosity of genes has little influence on bilateral asymmetry in turkeys.

The comparison of the selected and randombred control lines was complicated by a difference in body weight between the two groups of lines. The selected lines as a group had heavier body weight than the average of the three randombred controls. Therefore, the large-bodied (F, FL, and RBC3) and small-bodied lines (E, RBC1, and RBC2) were contrasted. The RA of shank length and shank depth in both sexes and shank width in females was larger in the large-bodied lines than in the small-bodied lines. There was no difference between the two groups of lines for RA of shank width of males and face length of both sexes. When the total or

Table 7. Total and Average Relative Asymmetry[1] of Shank Length, Shank Width,[2] Shank Depth[3] and Face Length[4] in Individual Turkey Lines and Groups of Lines.

Lines[5]	Males		Females	
	Total	Average	Total	Average
	(%)			
RBC1	12.1	3.0	11.3[b]	2.8[b]
E	11.3	2.8	9.6[b]	2.4[b]
RBC2	12.9	3.2	9.5[b]	2.4[b]
F	12.2	3.1	14.9[a]	3.7[a]
FL	13.2	3.3	15.3[a]	3.8[a]
RBC3	13.0	3.2	14.7[a]	3.7[a]
Randombred	12.7	3.2	11.8	3.0
Selected	12.2	3.0	13.3	3.3
Small-bodied	12.1	3.0	10.1[y]	2.5[y]
Large-bodied	12.8	3.2	15.0[x]	3.7[x]

[a-b] Individual line means within columns with no common superscript are significantly different ($P \leq 0.05$).

[x-y] Means within groups of lines in columns with no common superscript are significantly different ($P \leq 0.05$).

[1] ((| Right-left |) ÷ ((right + left) ÷ 2)) X 100.

[2] Width measured laterally at the dew claw.

[3] Width measured perpendicular at the dew claw

[4] Distance between auditory canal opening and the posterior junction of the upper and lower mandible.

[5] RBC1, RBC2, RBC3 = randombred control lines; E =s ubline of RBC1 selected for increased egg production; F = subline of RBC2 selected for increased 16-week BW; FL = subline of F selected for increased shank width; small-bodied = RBC1, E, and RBC2 lines; and large-bodied = F, FL, and RBC3 lines.

average RA for the four traits was analyzed, RA for males did not differ between the large-bodied and small-bodied lines but in females, RA was greater for the large-bodied lines. The males and females were reared in different houses and all lines were reared intermingled. The RA was higher for small-bodied males than for small-bodied females, while the reverse was true for the large-bodied lines. It is possible that the social competition was a disadvantage for small-bodied males and large-bodied females when the lines were reared intermingled. Overall, the results of the present study using turkeys indicated that body weight had a greater influence on developmental stability than homozygosity of genes but that not all bilateral traits are similarity affected. In chickens, Møller *et al.* (1995) reported that FA of several traits was greater in fast-growing breeds than in slower-growing ones.

Both FA and DA was observed in the present study. No AS was observed. In the individual line and sex subgroups, the bilateral differences exhibited FA in 38 of 48 comparisons. In some cases, FA was observed in one sex while DA was found in the other sex of the same line. In chickens, Yang *et al.* (1997) and Yang and Siegel (1998) observed that the type of asymmetry could vary within lines and AS was observed in their studies.

The traits were ranked face length > shank width = shank depth > shank length for RA in the present study. A similar ranking of shank length, shank depth, and face length was observed in chickens by Yang *et al.* (1998).

In summary, the RA of shank length, shank width, shank depth, and face length was measured in three randombred control and three long-term selected lines. The selected lines were selected for increased egg production, increased 16-week body weight, or increased shank width and had a higher level of inbreeding than the randombred control lines. Homozygosity as measured by a comparison of the randombred control and selected line had little influence on RA. A comparison of large-bodied and small-bodied lines indicated that body

Table 8. Average Relative Asymmetry[1] in Shank Length, Shank Width,[2] Shank Depth,[3] and Face Length[4] in Males and Females.

Trait	Males	Females
	(%)	
Shank length	0.95[c]	1.11[c]
Shank width	2.56[b]	3.22[b]
Shank depth	2.88[b]	2.96[b]
Face length	6.04[a]	5.25[a]

[a-b] Means within columns with no common superscript are significantly different ($P \leq 0.05$).

[1] ((| Right-left |) ÷ ((right + left)÷) 2)) X 100.

[2] Width measured laterally at the dew claw.

[3] Width measured perpendicular at the dew claw.

[4] Distance between auditory canal opening and the posterior junction of the upper and lower mandible.

weight had a greater influence than homozygosity on RA. Fluctuating asymmetry and DA, but not AS, were observed.

References

Clarke, G. M. 1998. The genetic basis of developmental stability. V. Inter-and intra-individual character variation. *Heredity* 80:562-567.

Havenstein, G. B., K. E. Nestor, V. D. Toelle, and W. L. Bacon. 1988. Estimates of genetic parameters in turkeys. 1. Body weight and skeletal characteristics. *Poultry Sci.* 67:1378-1387.

Johnson, A. S. and V. S. Asmundson. 1957. Genetic and environmental factors affecting size of body and body parts of turkeys. 1. The heritability and interrelationships of body weight and live body measurements. *Poultry Sci.* 36:296-301.

Leary, R. F. and F. W. Allendorf. 1989. Fluctuating asymmetry as an indicator of stress: Implications for conservation biology. *Trends Ecol. Evol.* 4:214-217.

Lerner, I. M. 1954. *Genetic Homeostasis.* John Wiley and Sons, New York, N.Y.

McCartney, M. G. 1964. A randombred control population of turkeys. *Poultry Sci.* 43:763-765.

McCartney, M. G., K. E. Nestor, and W. H. Harvey. 1968. Genetics of growth and reproduction in the turkey. 2. Selection for increased body weight and egg production. *Poultry Sci.* 47:981-990.

Møller, A. P., G. S. Sanotra, and K. S. Vestergaard. 1995. Developmental stability in relation to population density and breed of chickens *Gallus gallus*. *Poultry Sci.* 74:1761-1771.

Naber, E. C. and S. P. Touchburn. 1970. *Ohio Poultry Rations.* Ohio Cooperative Extension Service Bulletin 343. The Ohio State University, Columbus, Ohio.

Nestor, K. E. 1971. Genetics of growth and reproduction in the turkey. 3. Further selection for increased egg production. *Poultry Sci.* 50:1672-1682.

Nestor, K. E. 1977. Genetics of growth and reproduction in the turkey. 5. Selection for increased body weight alone and in combination with increased egg production. *Poultry Sci.* 56:337-347.

Nestor, K. E., W. L. Bacon, G. B. Havenstein, Y. M. Saif, and P. A. Renner. 1988. Carcass traits of turkeys from lines selected for increased growth rate or increased shank width. *Poultry Sci.* 67:1660-1667.

Nestor, K. E., W. L. Bacon, Y. M. Saif, and P. A. Renner, 1985. The influence of genetic increases in shank width on body weight, walking ability, and reproduction of turkeys. *Poultry Sci.* 64:2248-2255.

Nestor, K. E., M. G. McCartney, and N. Bachev. 1969. Relative contributions of genetics and environment to turkey improvement. *Poultry Sci.* 48:1944-1949.

Nestor, K. E., M. G. McCartney, and W. R. Harvey. 1967. Genetics of growth and reproduction in the turkey. 1. Genetic and non-genetic variation in body weight and body measurements. *Poultry Sci.* 46:1374-1384.

Nestor, K. E., D. O. Noble, J. Zhu, and Y. Moritsu. 1996. Direct and correlated responses to long-term selection for increased body weight and egg production in turkeys. *Poultry Sci.* 75:1180-1191.

Noble, D. O., D. A. Emmerson, and K. E. Nestor. 1995. The stability of three randombred control lines of turkeys. *Poultry Sci.* 74:1074-1078.

Palmer, A. R. 1996. Waltzing with asymmetry. *BioScience* 46:518-532.

Palmer, A. R. and C. Strobeck. 1986. Fluctuating asymmetry: Measurement, analysis, patterns. *Annu. Rev. Ecol. Syst.* 17:391-421.

Palmer, A. R. and C. Strobeck, 1992. Fluctuating asymmetry as a measure of developmental stability: Implications of non-normal distributions and power of statistical tests. *Acta Zool. Fenn.* 191:57-72.

Parsons, P. A. 1990. Fluctuating asymmetry: An epigenetic measure of stress. *Biol. Rev.* 65:131-145.

SAS Institute. 1988. *SAS/STAT User's Guide.* 6.03 Edition. SAS Institute, Inc., Cary, N.C.

Thoday, J. M. 1958. Homeostasis in a selection experiment. *Heredity* 12:401-415.

Van Valen, L. 1962. A study of fluctuating asymmetry. *Evolution* 16:125-142.

Yang, A., E. A. Dunnington, and P. B. Siegel. 1997. Developmental stability in stocks of White Leghorn chickens. *Poultry Sci.* 76:1632-1636.

Yang, A., E. A. Dunnington, and P. B. Siegel. 1998. Developmental stability in different genetic stocks of white rock chickens. *J. Hered.* 89:260-264.

Yang, A. and P. B. Siegel. 1998. Asymmetries and heterosis of bilateral traits in parental lines of chickens and their F_1 crosses. *J. Anim. Breed. Genet.* 113:105-111.

Effects of the Rendement Napole Gene: Muscle Quality and Breed Differences for High and Low Glycolytic Potential Groups in Swine

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Abstract

Improvement of meat quality is one of the primary concerns of the pork industry. Many genetic and environmental factors contribute to the quality of fresh and processed meat products. The dominant Rendement Napole gene (RN) has been found to have both positive and negative effects on pork quality. Currently, the best method for classification of animals as RN positive (RN^-RN^-, RN^-rn^+) or RN negative (rn^+rn^+) is the glycolytic potential test (GP). High glycolytic potential indicates that the animal is a carrier of the RN gene. This study investigates the effect of GP on pork quality traits for a population of 576 post-mortem *longissimus dorsi* samples from the 1998 National Barrow Show Progeny Test. Animals were classified as high glycolytic potential (n = 26) or low glycolytic potential (n = 550), based on a GP threshold of 160 µmoles lactate equivalents per gram for the population bimodal distribution. Objective muscle-quality traits measured included loin pH (pH), water-holding capacity (WHC) measured as the weight (mg) of exudate absorbed on a filter paper, Instron tenderness (INS), percent cooking loss (CL), and Minolta color (MIN). Sensory scores evaluated included tenderness (TEN) and juiciness (JUC). Residual correlations between GP and pH, INS, WHC, CL, and MIN were -0.55, 0.15, 0.20, 0.29, and 0.29, respectively. High GP pigs had significantly ($P < 0.01$) lower pH (5.42 vs. 5.57) and WHC (0.055 vs. 0.037), greater CL (22.0 vs. 19.2) and paler MIN color (25.24 vs. 23.02) than low GP pigs. No statistical differences were found between low and high GP pigs for INS, JUC, or TEN. Breed was a significant source of variation for all traits evaluated. Berkshire and Chester White breeds exhibited significantly ($P < 0.001$) lower GP values than Hampshire or Hampshire crossbred samples. The results of this study agree with previous research indicating that high GP values are associated with lower pH, poorer WHC, higher CL, and paler color. The differences in GP across breeds warrant future studies to determine the relationship of GP with muscle quality and sensory traits.

Introduction

In order to strengthen consumer acceptance, meat-quality improvement has recently become one of the top priorities of the pork industry. Many different environmental and genetic factors can influence the quality of fresh or processed pork products. Most of the past genetic research relating to pork quality has focused on the Halothane or "stress" gene, which is associated with pale, soft, and exudative (PSE) pork. Recently, the Rendement Napole (RN), or "acid meat" gene, has been shown in European and U.S. research to have both negative and positive effects on pork quality.

Carriers of the dominant RN gene have been shown to exhibit paler color, reduced pH and water-holding capacity, as well as increased drip

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and cooking losses (Monin and Sellier, 1985; Enfalt *et al.*, 1994; LeRoy *et al.*, 1996; Lundstrom *et al.*, 1996; Sutton, 1997; Enfalt *et al.*, 1997). Positive effects of increased tenderness, juiciness, growth, and carcass advantages have also been reported for Napole carriers (Enfalt *et al.*, 1994; LeRoy *et al.*, 1996; Sutton, 1997).

While PSE pork is attributed to a combination of higher temperature and a rapid rate of pH decline in muscle, the effects of the RN gene are the result of lower ultimate muscle pH. U.S. researchers, Sayre *et al.* (1963), were the first to report that Hampshire pigs had lower ultimate pH and paler color. However, these differences were not further investigated until Monin and Sellier (1985) reported that this lower ultimate pH was the result of high "glycolytic potential." Glycogen is the major source of energy in the muscle. When needed, glycogen is broken down through the glycolytic pathway, of which lactic acid is the end result in post-mortem muscle tissue (Figure 1).

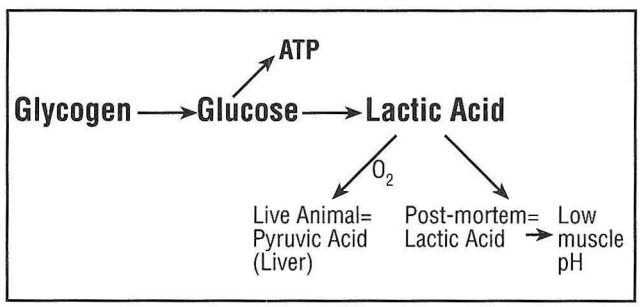

Figure 1. Simplified diagram of glycogen conversion in the muscle.

The RN gene is mainly associated with lines of Hampshire breeding, with reports of the gene frequency being as high as 0.627 in U.S. Hampshire populations (Miller, 1998). Given this high frequency within a popular terminal sire breed, there is great potential for increased economic losses from the undesirable pork-quality effects of the RN gene. Few studies on pigs with diverse genetic backgrounds have been completed for U.S. populations, which leaves open the possibility of the presence of RN in other breeds.

While the search for a DNA marker continues (Mariani *et al.*, 1996; Milan *et al.*, 1996), the best method of classifying animals as either RN positive (RN^-RN^- or RN^-rn^+) or RN negative (rn^+ rn^+), is the use of the glycolytic potential (GP) test (Monin and Sellier, 1985). Individuals that carry the dominant RN gene exhibit higher glycolytic potential. High or low GP classifications are based on a bimodal distribution threshold value unique to each population (Naveau, 1986).

The first phase of The Ohio State University Napole gene research involves the classification of a diverse population by GP testing, followed by statistical analysis of all correlated production, carcass, meat quality, and sensory traits. Correlations between GP and pork-quality traits of interest, as well as a study of progeny groups and pedigree information, will be completed. Differences between breeds for quality traits will also be reported.

Materials and Methods

Samples

Five hundred seventy-six frozen loin-muscle samples from animals previously characterized for growth, carcass, and meat quality characteristics in the 1998 National Barrow Show Progeny Test were obtained. Berkshire (n = 184), Chester White (n = 99), Duroc (n = 77), Hampshire (n = 22), Landrace (n = 55), Poland China (n = 24), Spot (n = 15), Yorkshire (n = 69), Hampshire crossbred (n = 16), Poland x Duroc F1 crossbred (n = 8), and Tamworth x Hampshire crossbred (n = 7) were represented in the test. All animals were subjected to similar on-test management, carcass evaluation, and sensory analysis.

Glycolytic Potential Test

Glycolytic potential of the loin muscle is the estimated sum of the compounds that have the potential to be transformed into lactic acid in the post-mortem muscle. GP (μmole/g) is calculated as 2([glycogen] + [glucose-6-phosphate] + [glucose]) + [lactate]. Loin samples were subjected to a preparation procedure developed by Dalrymple and Hamm (1973), which allows for the simultaneous extraction of metabolites of interest from the muscle. The concentration of glycogen, glucose, and glucose-6-phosphate was determined by a procedure developed by Keppler and Decker (1972), while the amount of lactate present in the muscle was determined according to the procedures of Bergmeyer (1974). Animals were classified as high glycolytic potential (n = 26) or low

glycolytic potential (n = 550) based on a GP threshold of 160 µmoles lactate equivalents per gram for the population bimodal distribution.

Statistical Analysis

Muscle quality and sensory traits were analyzed in a mixed model analysis of SAS (1988) with fixed effects of day off test, breed, and GP status and a random sire (breed) effect. Partial correlation coefficients were calculated among quality traits using the MANOVA statement in SAS (1988).

Results and Discussion

Residual correlations ($P < 0.01$) between GP and pH, INS, WHC, CL, and MIN were -0.55, 0.15, 0.20, 0.29, and 0.29, respectively (Table 1). As expected, pH had the strongest relationship and was negatively correlated with GP. This is in agreement with Miller (1998) who also found a negative correlation coefficient of -0.49 for GP and longissimus pH. These results show that as glycolytic potential increases, so does tenderness and cooking loss, while pH and water-holding capacity decrease.

High GP pigs had significantly ($P < 0.01$) lower pH (5.42 vs. 5.57) and WHC (0.055 vs. 0.037), greater CL (22.0 vs. 19.2) and paler MIN color (25.24 vs. 23.02) than low GP pigs (Table 2). These results are also in agreement with previous research (Lundstrom *et al.*, 1996; Sutton, 1997; Enfalt *et al.*, 1997) showing that the high GP samples lose more water than low GP samples. No statistical differences were found in this study between low and high GP pigs for INS, JUC, or TEN, indicating no advantage in tenderness or juiciness for the high glycolytic potential group as was reported by both Miller (1998) and Lundstrom *et al.* (1996).

Breed was a significant source of variation for all traits evaluated (Table 3). Berkshire (Berk) and Chester White (Chester) breeds exhibited significantly ($P < 0.001$) lower GP values than Hampshire (Hamp) or Hampshire crossbreed (Hamp-X) samples. However, Hamp GP was not different ($P > 0.05$) from Hamp-X, Yorkshire (York), Landrace, Spot, or Tam/Hamp-X. Berk and Chester loin pH were also significantly higher than both the Hamp and Hamp-X breeds. Berks exhibited the most desirable WHC and CL when compared with the other breeds represented. Hamp and Hamp-X were only different in WHC from Berk and Chester. Landrace had the least desirable water-holding capacity but were not significantly different than Poland, York, Hamp-X, Spot, Poland-X, or Tam/Ham-X. Landrace also had the highest cooking losses but were only different from Hamp, Chester,

Table 1. Correlations Between Glycolytic Potential and Pork Quality Characteristics.[1]

	pH***	WHC***	INS**	CL***	MIN***
Glycolytic Potential	-0.55	0.20	0.15	0.29	0.29

*** Significant at $P < 0.001$, **Significant at $P < 0.01$.

[1] pH = Ultimate Loin pH, WHC = Water Holding Capacity (mg of exudate), INS = Instron tenderness, CL = Cooking Loss (%), MIN = Minolta reflectance

Table 2. Least Squares Means of High and Low Glycolytic Potential Groups for Pork Quality Characteristics.[1]

Group[2]	n	GP***	pH***	WHC***	INS	CL**	MIN**	TEN	JUC
High GP	26	177.32	5.42	0.055	4.92	22.00	25.24	6.8	5.29
Low GP	550	110.21	5.57	0.037	5.29	19.22	23.02	6.9	5.35

*** Significant at $P < 0.001$, **Significant at $P < 0.01$.

[1] GP = Glycolytic Potential (µmole/g), pH = Ultimate Loin pH, WHC = Water Holding Capacity (mg of exudate), INS = Instron tenderness, CL = Cooking Loss (%), MIN = Minolta reflectance, TEN = Tenderness Sensory Score (1 = extremely tough), JUC = Juiciness Sensory Score (1 = extremely dry).

[2] High GP = greater than 160 µmole/g; Low GP = less than 160 µmole/g.

Table 3. Least Squares Means of Breeds for Pork Quality Characteristics.[1]

Breed	n	GP	pH	WHC	CL	TEN
Berkshire	184	131.93	5.61	0.038	18.13	7.35
Chester	99	132.09	5.60	0.039	20.12	6.35
Duroc	77	141.45	5.51	0.045	21.21	6.46
Hampshire	22	150.40	5.48	0.045	20.27	7.57
Hamp-X	16	151.11	5.46	0.045	20.57	7.99
Landrace	55	151.03	5.44	0.056	22.48	6.91
Poland	24	142.59	5.49	0.051	20.86	6.20
Poland-X	8	132.99	5.53	0.049	19.12	5.71
Spot	15	148.88	5.39	0.046	21.50	6.55
Tam/Hamp-X	7	147.51	5.51	0.042	21.43	6.99
Yorkshire	69	151.47	5.41	0.049	20.96	7.04

[1] GP = Glycolytic Potential (μmole/g), pH = Ultimate Loin pH, WHC = Water-Holding Capacity (mg of exudate), CL = Cooking Loss (%), TEN = Tenderness Sensory Score (1 = extremely tough).

Poland-X, Berk, and York. Hamp and Hamp-X were scored the most tender but were not significantly different from Berk, York, Landrace, or Tam/Hamp-X.

Trends within the breeds for the analysis closely follow those previously reported in past investigations (Goodwin, 1997). However, the limited number of high GP Hampshires in this analysis may lead to results that are not characteristic of the actual breed differences attributed to effects of the RN gene. Another factor to consider is that Hampshires did not contribute all of the 26 individuals classified as high GP, which suggests evidence of the presence of the RN gene in other breeds. Further investigations into these breed differences will be conducted.

Conclusions

The results of this study agree with previous research indicating that high glycolytic potential values are associated with lower loin ultimate pH, poorer water-holding capacity, higher cooking losses, and paler color. Berkshire and Chester White samples were higher in pH and lower in glycolytic potential than both the Hampshire and Hampshire crossbred samples. However, the differences across breeds for the other quality traits studied warrant future investigations to determine the relationship of GP with muscle quality and sensory traits.

References

Bergmeyer, H. U. 1974. *Methods of Enzymatic Analysis.* Academic Press, N.Y.

Dalrymple, R. H. and R. Hamm. 1973. A method for the extraction of glycogen and metabolites from a single muscle sample. *J. Food. Technol.* 8:439.

Enfalt, A. C., K. Lundstrom, L. Lundkvist, A. Karlsson, and I. Hansson. 1994. Technological meat quality and the frequency of the RN-gene in purebred Swedish Hampshire and Yorkshire pigs. 40th IcoMST. The Haque, Netherlands.

Enfalt, A. C., K. Lundstrom, I. Hansson, S. Johansen, and P. Nystrom. 1997. Comparison of noncarriers and heterozygous carriers of the RN-allele for carcass composition, muscle distribution, and technological meat quality in Hampshire-sired pigs. *Livestock Prod. Sci.* 47:221.

Goodwin, R. 1997. *Genetic Effects on Pork Quality.* 1997 Pork Quality Summit. Des Moines, Iowa.

Keppler, D. and K. Decker. 1972. Glycogen determination with amyloglucosidase. *In: Methods of Enzymatic Analysis.* Vol II. p 1127. Academic Press, N.Y.

LeRoy, P., G. Monin, J. M. Elsen, J. C. Cartiez, A. Talmant, B. Lebret, L. Lefaucheur, J.Mourot, H. Juin, and P. Sellier. 1996. Effect of the RN genotype on growth and carcass traits in pigs. 47th EAAP. Lillehammer, France.

Lundstrom, K., A. Anderson, and I. Hansson. 1996. Effect of the RN gene on technological and

sensory meat quality in crossbred pigs with Hampshire as terminal sire. *Meat Sci.* 42:145.

Mariani, P., K. Lundstrom, U. Gustafasson, A. C. Enfalt, R. K. Juneja, and L. Andersson. 1996. A major locus (RN) affecting muscle glycogen content is located on pig chromosome 15. *Mammalian Genome.* 7:52.

Milan, D., N. Woloszyn, M. Yerle, P. LeRoy, M. Bonnet, J. Riquet, Y. Lahbib-Mansais, J. C. Caritez, A. Robic, P. Sellier, J. M. Elsen, and J. Gellin. 1996. Accurate mapping of the "acid meat" RN gene on genetic and physical maps of pig chromosome 15. *Mammalian Genome* 7:47.

Miller, K. D. 1998. The detection and characterization of pigs with differing glycolytic potential levels within the United States swine populations. Ph.D. Dissertation. University of Illinois, Urbana-Champaign.

Monin, G. and P. Sellier. 1985. Pork of low technological quality with a normal rate of muscle pH fall in the immediate post-mortem period: The case of the Hampshire breed. *Meat Sci.* 13:49.

Naveau, J. 1986. Contribution a l'etude du determinisme genetique de la qultie de la viand porcine. Heritabilite du redement technologique Napole J. Rech. *Porcine en France.* 18:265.

SAS. 1988. *SAS/STAT User's Guide.* Release 6.03. SAS Inst., Inc., Cary, N.C.

Sayre, R. N., E. J. Briskey, and W. G. Hoekstra. 1963. Comparison of muscle characteristics and postmortem glycolysis in three breeds of swine. *J. Animal. Sci.* 22;1012.

Sutton, D. S. 1997. The influence of the Rendement Napole gene on pork quality. Ph.D. Dissertation. University of Illinois, Urbana-Champaign.

The Effect of the Estrogen Receptor Gene on Litter Traits in Swine

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Abstract

The identification of genes or markers associated with reproductive traits in swine is an important area of research, due to the large economic impact that these discoveries could have on the swine industry. It has previously been reported that one of these genes, the estrogen receptor (ESR) gene, is associated with increased litter size in pigs. There has been little research, however, on the association between the ESR gene and other litter traits such as number of piglets alive at weaning. The purpose of this study was to investigate the association between ESR genotype and these litter traits in a population of 322 Large White, Yorkshire, and crossbred pigs. Two hundred twelve litter records from these animals were collected and analyzed for associations between ESR genotype and the following litter traits — number born, number born alive, litter weight of animals born, litter weight of animals born alive, number of mummies, number of stillborn animals, number of overlaid animals, number of animals at weaning, and litter weight at weaning. Data were analyzed using a model that included the effects of ESR genotype of dam, parity, farrowing month, dam breed, sire breed, and significant two-way interactions. Some litter traits displayed favorable, but not statistically significant, trends with respect to ESR genotype — litter weight born alive, litter weight born, number of stillborn pigs, number of pigs at weaning, and total litter weight at weaning. Paternal and maternal breed effects were also found for several of the litter traits studied. Dams with Large White fathers had an increased number of stillborn piglets (P = 0.08) and an increased number of mummies (P = 0.07). Dams with Large White mothers had an increased number of piglets alive at weaning (P = 0.10) and an increased litter weight of piglets alive at weaning (P = 0.001).

Introduction

There has recently been intense effort within the field of genetics to find the genes that control all aspects of physiology. Special attention has been focused on those genes that are thought to control the physiological pathways that influence the ability of our common livestock species to produce meat and milk. In the past, these pathways were manipulated by selecting superior animals for use in traditional breeding programs. Unfortunately, many of these physiological traits cannot be quickly or easily improved using traditional methods of selection. In these cases, the newly expanded field of molecular genetics presents a solution. If specific regions of the genome (termed "markers") that have a direct effect on physiological traits are isolated, animals containing these markers can then be selected for using marker-assisted selection schemes. Marker-assisted selection holds great promise to help improve the rate of selection for traits that are reproductive in nature. In swine, special attention has been placed on the discovery of markers that influence the ability of a mother to successfully give birth and care for her young. Traits that influence this ability are termed the litter traits. Litter traits, such as number born and number of piglets alive at weaning, are characteristically of low heritability (Rothschild and Bidanel, 1998). This makes their improvement us-

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ing marker-assisted selection techniques especially attractive.

One of the first markers shown to have a significant association with litter traits in swine was the estrogen receptor (ESR) gene (Rothschild *et al.*, 1991). Previous studies have shown that females with the favorable ESR B allele have an advantage of +0.3 (Large White) to +1.2 (Meishan) pigs born per litter over those females that do not have the favorable allele (Rothschild *et al.*, 1994; Short *et al.*, 1997). Little research has focused on the association between the ESR gene and other litter traits (such as number of pigs at weaning and litter weight at weaning), however. The purpose of this current study was to determine the relationship between these uninvestigated litter traits and the ESR gene.

Materials and Methods

Animals

Three hundred twenty-two purebred Yorkshire (Y x Y), purebred Large White (LW x LW), and crossbred (LW x Y, Y x LW) animals were selected for use in this study. All animals were raised at the Western Branch of The Ohio State University's Ohio Agricultural Research and Development Center (South Charleston, Ohio). Animals consisted of related and unrelated animals of both sexes and varying ages

ESR Genotype Analysis

For each animal, DNA was extracted from lymphocytes and the ESR gene amplified using a polymerase chain reaction protocol. This protocol has been outlined previously (Short *et al.*, 1997). Amplified products were digested with *PvuII* restriction endonuclease, separated on a 4% agarose gel, and visualized under UV light after ethidium bromide staining. Two ESR alleles (A and B) were identified and each animal was classified as either AA, AB, or BB with respect to ESR genotype.

Litter Data Collection

Litter data for all dams with a known ESR genotype were obtained and included in litter data analysis. Dams consisted of all four breed combinations and a variety of parities. Sires of litters were either purebred Yorkshire, purebred Large White, or purebred Hampshire in origin. Litter data were collected for farrowing seasons ranging from August 1994 to July 1998. In total, 212 litter records were collected and included in the analysis. Data subsequently included in the analysis were ESR genotype, farrowing month, sire breed, dam breed, number born, number born alive, litter weight of animals born, litter weight of animals born alive, number of mummies, number of stillborn animals, number of overlaid animals, number of animals at weaning, number of days to weaning, and litter weight at weaning. Weaning age ranged from five to 26 days, with an average weaning age of 20 days. Number of overlaid animals was defined as the number of piglets crushed by the dam. Number born was defined as the number of viable animals born plus the number of stillborn animals. Number born alive was defined as the number of viable animals born.

Statistical Analysis

All reproductive tract data were analyzed using the General Linear Model Procedures of SAS (1990). Data were analyzed using a model that included the effects of ESR genotype of dam, parity, farrowing month, dam breed, sire breed, and significant two-way interactions. Weaning age was included as a covariant in all models involving weaning data. Linear contrasts were used to determine the presence of individual heterosis, maternal breed effects, and paternal breed effects.

Results and Discussion

The fit of the model for most litter traits was poor, with low R2 values and non-linear normal probability plots (data not shown). This is probably a result of the interaction between complex environmental effects and fetal genotypes on litter traits. The poor fit of these models seriously hampers the interpretation of results from the litter data analysis. Increasing the number of animals in the study would assist in the determination of the validity of all trends and their true significance.

The main effect of ESR genotype was not significant ($P < 0.05$) for any of the traits analyzed. There was an ESR genotype x breed of dam interaction for both litter weight of pigs born alive ($P = 0.05$) and litter weight of pigs born ($P = 0.08$). In both of these cases, Y x Y animals with the BB genotype were found to have lower values than other ESR genotype x breed of dam combinations.

The ESR genotype also displayed notable, but not statistically significant, trends with respect to

Table 1. Least-Squares Means, Standard Errors, and P-Values for Selected Litter Traits.

Litter Trait[a]	N	Least-Squares Means and Standard Errors for Dams With Specified ESR Genotype			P-Value[b]
		AA	AB	BB	
ALIVEWT	212	14.64 ± 0.78	13.23 ± 0.57	12.60 ± 1.18	0.16
BORNWT	211	15.35 ± 0.69	14.77 ± 0.52	14.32 ± 0.91	0.53
NOSTILL	212	1.36 ± 0.28	1.11 ± 0.23	0.98 ± 0.35	0.49
NOWEAN	204	8.12 ± 0.48	8.20 ± 0.37	8.61 ± 0.59	0.70
WEANWT	203	47.4 ± 2.5	47.6 ± 1.9	50.3 ± 3.1	0.59

[a] ALIVEWT = total litter weight of animals born alive (kg), BORNWT = total litter weight of animals born (kg), NOSTILL = number of stillborn animals, NOWEAN = number of piglets alive at weaning, WEANWT = total litter weight at weaning (kg)

[b] Significance level of effect of ESR genotype on specified trait

Table 2. Least-Squares Means, Standard Errors, and P-Values Used in the Determination of Breed Effects for Litter Traits.

Breed of Dam[a]	Least-Squares Means and Standard Errors for Selected Litter Traits[b]			
	NOSTILL	NOMUM	NOWEAN	WEANWT
YxY	0.71 ± 0.22	0.043 ± 0.071	7.82 ± 0.37	43.3 ± 2.1
LWxLW	1.31 ± 0.26	0.284 ± 0.082	8.41 ± 0.43	53.3 ± 2.6
YxLW	1.16 ± 0.38	0.090 ± 0.121	8.89 ± 0.63	52.1 ± 3.5
LWxY	1.41 ± 0.40	0.137 ± 0.126	8.11 ± 0.67	44.9 ± 3.7
Maternal Breed Effect P-value[c]	0.50	0.22	0.10	0.001
Paternal Breed Effect P-value[d]	0.09	0.07	0.82	0.59

[a] YxY = Yorkshire sire x Yorkshire dam, YxLW = Yorkshire sire x Large White dam, LWxLW = Large White sire x Large White dam, LWxY = Large White sire x Yorkshire dam.

[b] NOSTILL = number of stillborn animals, NOMUM = number of mummified animals, NOWEAN = number of piglets alive at weaning, WEANWT = total litter weight at weaning (kg).

[c] For the linear contrast for maternal effects, where H_0 = no differences in the indicated trait between animals with the same breed of dam.

[d] For the linear contrast for paternal effects, where H_0 = no differences in the indicated trait between animals with the same breed of sire.

certain litter traits (Table 1). Animals with additional B alleles tended to have heavier litters at both birth and weaning. The hypothesized Chinese origin of the ESR B allele would explain this result, as Chinese pigs are known to have greater litter size and postnatal survival than domestic breeds (Haley *et al.*, 1995).

Paternal breed effects (Table 2) were observed for both number of stillborn piglets (P = 0.09) and number of mummies (P = 0.07). Dams with Large White fathers had both a larger number of stillborn piglets and number of mummies than dams with Yorkshire fathers. But, the poor fit of the number of stillborn piglets (R^2 = 0.103) and number of mummies (R^2 = 0.062) models seriously reduces the interpretability of these results.

Maternal breed effects (Table 2) were observed for both number of piglets alive at weaning (P = 0.10) and litter weight of piglets alive at weaning (P = 0.001). Dams with Large White mothers had a larger number of piglets alive at weaning and a larger litter weight of piglets alive at weaning than dams with Yorkshire mothers. Individual heterosis was not detected for any of the traits studied (data not shown).

Conclusion

From this study, it appears that the ESR gene may be associated with several new litter traits, such as number of piglets at weaning and litter weight at weaning. ESR genotype did not significantly affect any of the traits studied. However, several of the traits did show favorable, but non-significant, trends with respect to ESR genotype. The addition of more litter records to this analysis in the future will help validate and explain the significance of these trends.

References

Haley, C. S., E. D'Agaro, and M. Ellis. 1992. Genetic components of growth and ultrasonic fat depth traits in Meishan and Large White pigs and their reciprocal crosses. *Animal Production.* 266:105.

Rothschild, M. F., R. Larson, C. Jacobson, and P. Pearson. 1991. *PvuII* Polymorphisms at the Porcine Oestrogen Receptor Locus (ESR). *Anim. Genet.* 22:448.

Rothschild, M., C. Jacobson, D. Vaske, C. Tuggle, L. Wang, T. Short, G. Eckardt, S. Sasaki, A. Vincent, D. McLaren, O. Southwood, H. van der Steen, A. Mileham, and G. Plastow. 1996. The estrogen receptor locus is associated with a major gene influencing litter size in pigs. *Proc. Natl. Acad. Sci. USA.* 93:201.

SAS. 1990. *SAS/STAT User's Guide.* SAS Institute, Inc. Cary, N.C.

Short, T. H., M. Rothschild, O. Southwood, D. McLaren, A. de Vries, H. van der Steen, G. Eckardt, C. Tuggle, J. Helm, D. Vaske, A. Mileham, and G. Plastow. 1997. Effect of the estrogen receptor locus on reproduction and production traits in four commercial pig lines. *J. Anim. Sci.* 75:3138.

Rothschild, M. F. and J. P. Bidanel. 1998. Biology and genetics of reproduction. *In: The Genetics of the Pig.* M. F. Rothschild and A. Ruvinsky, Ed. pp. 313-343. CAB International, New York, N.Y.

Examination of the Relationship Between the Estrogen Receptor Gene and Reproductive Tract Components in Swine

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Abstract

Previous studies have shown that a specific allele (B allele) of the estrogen receptor (ESR) locus is associated with increased litter size in swine. At this time, research is lacking in the examination of the association between ESR genotype and the reproductive system itself. The objective of this current study was to investigate the association between ESR genotype and reproductive components in swine. The ESR genotype of 322 Yorkshire (Y¥Y), Large White (LW¥LW), and crossbred (LWxY, YxLW) animals was determined to be either AA, AB, or BB using a PCR-RFLP procedure. Of this group, 107 females were selected and mated to Hampshire boars. At approximately day 75 of gestation, the females were slaughtered and their reproductive tracts collected. Data collected included ovulation rate, horn length, number of fetuses, fetal mass, uterine mass, number of mummies, fetal sex, fetal placement, fetal survival, and fetal space. Data were analyzed using a model that included ESR genotype, breed, parity, and significant two-way interactions. Uterine horn was also included in some analyses. ESR genotype was not found to be a significant ($P > 0.05$) effect for any of the traits studied. Some traits displayed favorable, but not statistically significant, trends with respect to ESR genotype — fetal survival, total uterine length, total fetal weight, total number of mummies, fetuses per horn, horn length, and fetal space. The ESR gene, therefore, appears to be positively associated with several reproductive traits. Parity and breed also affected some reproductive traits. Animals of parity ≥ 3 had both a significantly ($P < 0.05$) larger ovulation rate per horn (+1.78 ova) and a lower fetal space per horn (-16.01 cm) than animals of parity 1. Also, animals with a Large White dam had an increased number of fetuses per horn, increased fetal weight per horn, and a decreased fetal space per horn.

Introduction

For many years, scientists and producers alike have made tremendous improvements in our common livestock species using traditional methods of genetic selection. New discoveries in the field of molecular genetics now allow for the isolation and study of specific regions of the genome that influence important traits. Animals that contain these "marker" regions can then be selected for inclusion in a marker-assisted selection program. This approach has shown special promise for those traits that are of low heritability and act in a sex-limited manner, such as the reproductive traits. Due to the large part reproductive traits play in determining the efficiency of production in livestock species, a great deal of research has focused on the search for genes that influence these traits. An especially promising group of genes that has been investigated are those genes that are associated with the steroid hormones. The role of estrogen and the estrogen receptor in reproduction has been especially well studied. It has been shown

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that mutations in the estrogen receptor gene (ESR) can produce considerable phenotypic changes in the mammalian reproductive system, including cancer (Lehrer *et al.*, 1990) and infertility (Korach, 1994). Based on observations such as these, it was hypothesized that the ESR gene could influence reproductive traits in swine.

Initial studies of the ESR gene in swine utilized animals of the Chinese Meishan breed. The Meishan breed is historically known for its large litter sizes (Haley *et al.*, 1992). Studies using Meishan and Meishan crosses discovered variation at the ESR locus in these swine (Rothschild *et al.*, 1991). Subsequent studies have found an association between a favorable ESR allele and reproductive traits in several breeds of swine. This advantageous allele has a positive additive effect on total number born and number born alive in swine. The effect of this allele has been shown to range from 1.25 pigs/litter in Meishan crosses to 0.4 to 0.6 pigs/litter in Large White and Large White crosses (Rothschild *et al.*, 1994; Short *et al.*, 1997). Researchers have also tried to find an association between this locus and other traits in swine, such as backfat depth and teat number (Rothschild *et al.*, 1994; Short *et al.*, 1997). One area that has not been studied, however, is the association between the ESR gene and reproductive tract traits. If the ESR gene influences traits such as litter size, it should follow that this gene also influences the reproductive system itself. The purpose of this study is to determine the effect of the ESR gene on several of these reproductive tract traits in swine.

Materials and Methods

Animals

Three hundred twenty-two purebred Yorkshire (Y x Y), purebred Large White (LW x LW), and crossbred (LW x Y, Y x LW) animals were selected for use in this study. All animals were raised at the Western Branch of The Ohio State University's Ohio Agricultural Research and Development Center (South Charleston, Ohio). Animals consisted of related and unrelated animals of both sexes and varying ages.

ESR Genotype Analysis

For each animal, DNA was extracted from lymphocytes and the ESR gene amplified using a polymerase chain reaction protocol. This protocol has been outlined previously (Short *et al.*, 1997). Amplified products were digested with *PvuII* restriction endonuclease, separated on a 4% agarose gel, and visualized under UV light after ethidium bromide staining. Two ESR alleles (A and B) were identified, and each animal was classified as either AA, AB, or BB with respect to ESR genotype.

Reproductive Tract Collection and Analysis

Of the original 322 animals genotyped, 107 females were selected for reproductive tract analysis. Females selected were of all four breed combinations and varying parities. All females were bred to Hampshire boars. All females were slaughtered at approximately 75 days of gestation in a commercial slaughter facility. Animals were slaughtered in four separate groups, with approximately 30 animals in each group. Following slaughter, gravid uterine tracts were collected and analyzed. Data collected on these tracts included ovulation rate, horn length, number of fetuses in each horn, fetal weight, uterine weight, number of mummies, fetal sex, fetal placement, fetal survival ([number of fetuses / ovulation rate] * 100), and fetal space (uterine length / [number of fetuses + number of mummies]).

Statistical Analysis

Allele frequency analysis was performed using Excel 6.0. Allele and genotype frequencies were calculated within each of the breed subgroups and within each of the two larger (n = 322 and n = 107) groups. Expected genotype frequencies were calculated based on the Hardy-Weinberg equation. Expected and observed values were compared using a chi-square test to determine the presence of Hardy-Weinberg equilibria in each studied population.

All reproductive tract data were analyzed using the General Linear Model Procedures of SAS (1990). Data were analyzed using a model that included the effects of ESR genotype, breed, parity, and all significant two-way interactions. Uterine horn was also included in some analyses. Linear contrasts were used to determine the presence of individual heterosis and maternal breed effects.

Results and Discussion

Allele and genotype frequencies for the popu-

lation of slaughtered animals are shown in Table 1. The A allele was more frequent than the B allele in all breed groups except for the LW x Y group. All breed groups were also tested for the presence of Hardy-Weinberg equilibria (data not shown). All groups were found to be in equilibrium, except for the Y x LW group; this group had a larger number of animals with the AB genotype than was expected based on the Hardy-Weinberg principle. The small number of animals in each of the breed groups, however, makes the determination of Hardy-Weinberg equilibria very difficult; in small groups such as these, the effects of sampling error could be quite large.

ESR genotype was not found to be a significant ($P > 0.05$) effect for any of the traits studied. P-values for the effect of ESR genotype in the model were generally very high, ranging from 0.2 to 0.8. Some traits did show notable, but statistically non-significant, trends with respect to ESR genotype (Table 2). Note the trend for animals with additional copies of the ESR B allele to have increased fetal survival, increased fetal weight, increased uterine length, and decreased fetal space. These trends agree with previous reports that have showed the ESR B allele to be associated with an increased number of pigs born per litter. To determine these effects, however, large numbers of lit-

Table 1. Estrogen Receptor Gene (ESR) Allele and Genotype Frequencies for the Slaughtered Animal Population.

Breed of Animal[a]	N	ESR Allele Frequencies		ESR Genotype Frequencies		
		A	B	AA	AB	BB
YxY	36	0.51	0.49	0.22	0.58	0.19
YxLW	26	0.52	0.48	0.12	0.81	0.10
LWxLW	28	0.64	0.36	0.36	0.57	0.07
LWxY	17	0.38	0.62	0.06	0.65	0.29
Total Population	107	0.53	0.47	0.20	0.65	0.15

[a] YxY = Yorkshire sire x Yorkshire dam, YxLW Yorkshire sire x Large White dam, LWxLW = Large White sire x Large White dam, LWxY = Large White sire x Yorkshire dam.

Table 2. Least-Squares Means and Standard Errors for All Reproductive Traits That Showed Notable Trends With Respect to the Estrogen Receptor Gene (ESR) Genotype.

Reproductive Trait[a]	N	Least-Squares Means and Standard Errors for Animals With Specified ESR Genotype			P-Value[b]
		AA	AB	BB	
FETSRV	100	52.8 ± 4.7	59.1 ± 2.5	61.3 ± 5.2	0.38
TNOFET	100	10.20 ± 0.83	11.05 ± 0.44	11.58 ± 0.92	0.50
UTLTH	100	543 ± 23	567 ± 12	582 ± 25	0.48
TNOMUM	106	0.27 ± 0.15	0.27 ± 0.07	0.57 ± 0.22	0.45
TFETWT	100	3,735 ± 158	3,889 ± 87	4,004 ± 172	0.49
HNLTH	204	271.3 ± 8.4	282.5 ± 4.5	290.6 ± 9.1	0.27
NOFET	211	5.11 ± 0.30	5.57 ± 0.16	5.84 ± 0.34	0.23
FETSPC	204	58.3 ± 3.8	54.9 ± 1.9	52.1 ± 5.5	0.61

[a] FETSRV = percentage of ova per uterus that survive to day 75 of gestation, TNOFET = number of fetuses per uterus, UTLTH = total length of uterine horns (cm), TNOMUM = total number of mummies per uterus, TFETWT = total fetal weight (g) per uterus, HNLTH = length of uterine horn (cm), NOFET = number of fetuses per horn, FETSPC = amount of uterine space (cm) available per fetus.

[b] Significance level of effect of ESR genotype on specified trait.

ter records were required. In a study by Short *et al.* in 1997, more than 9,000 litter records were required in order to find the small effect of the ESR B allele. In contrast, our current study only utilized 107 records to determine the effect of the ESR gene. Therefore, it seems logical that observed trends were not found to be significant. The future addition of more animals to the study will help verify the validity of these trends.

Parity significantly affected several of the reproductive traits (Table 3). Animals of a higher parity have both a larger ovulation rate and a lower fetal space. This could reflect the increased reproductive efficiency of older animals, which ovulate more eggs, carry more piglets to farrowing, and have a reduced fetal space (Hughes and Varley, 1980). However, parity was not significantly associated with an increased number of fetuses (TNOFET, P = 0.220; NOFET, P = 0.0925).

Breed was also significantly associated with several of the traits studied (Table 4). Linear contrasts detected the presence of a maternal breed effect for several traits (Table 5). Animals with a Large White dam had an increased number of fetuses per horn, increased fetal weight per horn, and a decreased fetal space per horn. Linear contrasts did not detect the presence of positive individual heterosis for any of the traits studied. However,

Table 3. Least-Squares Means and P-Values for Reproductive Traits Associated With Parity.

Reproductive Trait[c]	N	Least-Squares Means for Animals of Specified Parity			P-value[d]
		1	2	≥ 3	
TOV	100	17.98[a]	18.49[a]	21.65[b]	0.0006
OV	204	9.00[a]	9.21[a]	10.78[b]	0.055
FETSPC	204	61.6[a]	58.0[ab]	45.6[b]	0.044

[a-b] Means within a row without a common subscript are significantly different (P < 0.05).

[c] TOV = total number of corpora lutea per animal, OV = number of corpora lutea per horn, FETSPC = amount of uterine space (cm) available per fetus per horn.

[d] Significance level of effect of parity on specified trait.

Table 4. Least-Squares Means and P-Values for Reproductive Traits Associated With Breed.

Reproductive Trait[d]	N	Least-Squares Means for Animals of Specified Breed[e]				P-value[f]
		YxY	YxLW	LWxLW	LWxY	
UTLTH	100	598[a]	548[b]	586[ab]	522[b]	0.036
TFETSPC	100	61.8[a]	50.8[b]	50.5[b]	56.3[ab]	0.054
HNLTH	204	298.9[a]	274.6[bc]	292.1[ab]	260.4[c]	0.002
FETWT	211	1,839[a]	1,949[b]	1,993[b]	1,731[a]	0.0001
FETSPC	204	62.3[a]	50.3[b]	50.6[b]	57.1[ab]	0.006

[a-c] Means within a row without a common subscript are significantly different (P < 0.05).

[d] UTLTH = total length of both uterine horns (cm), TFETSPC = amount of uterine space (cm) available per fetus per uterus, HNLTH = length of uterine horn (cm), FETWT = total fetal weight (g) per horn, FETSPC = amount of uterine space (cm) available per fetus per horn.

[e] YxY = Yorkshire sire x Yorkshire dam, YxLW = Yorkshire sire x Large White dam, LWxLW = Large White sire x Large White dam, LWxY = Large White sire x Yorkshire dam.

[f] Significance level of effect of parity on specified trait.

Table 5. Least-Squares Means and P-Values for the Determination of Breed Effects for Reproductive Traits.

Breed of Animal[a]	Least-Squares Means and Standard Errors for Selected Reproductive Traits[b]				
	TFETSPC	HNLTH	NOFET	FETWT	FETSPC
YxY	61.84 ± 3.18	298.9 ± 6.3	5.35 ± 0.23	1839 ± 38	62.33 ± 3.0
LWxLW	50.53 ± 3.89	292.1 ± 7.5	5.80 ± 0.27	1993 ± 44	50.63 ± 4.5
YxLW	50.84 ± 4.02	274.6 ± 8.0	5.94 ± 0.29	1949± 43	50.29 ± 3.5
LWxY	56.33 ± 4.85	260.4 ± 9.5	4.94 ± 0.34	1731 ± 53	57.07 ± 4.3
Pures versus Crosses P-value[c]	0.50	0.0002	0.31	0.06	0.38
Maternal Breed Effect P-value[d]	0.03	0.63	0.007	0.0001	0.004

[a] YxY = Yorkshire sire x Yorkshire dam, YxLW = Yorkshire sire x Large White dam, LWxLW = Large White sire x Large White dam, LWxY = Large White sire x Yorkshire dam.

[b] TFETSPC = amount of uterine space (cm) available per fetus per uterus, HNLTH = length of uterine horn (cm), NOFET = number of fetuses per horn, FETWT = total fetal eight (g) per horn, FETSPC = amount of uterine space (cm) available per fetus per horn.

[c] For the linear contrast for heterosis, where H_0 = no differences in the indicated trait between purebred and crossbred animals.

[d] For the linear contrast for maternal effects, where H_0 = no differences in the indicated trait between animals with the same breed of dam.

negative heterosis was detected for several of the traits (Table 5), with the purebred animals having a greater performance than the crossbred animals. Possibly, the low number of crossbred animals (compared to purebred) included in the study may affect the true determination of heterosis.

Conclusion

The results of this study allow us to begin to construct a preliminary picture of how the ESR gene positively influences the reproductive performance of the female pig. Adding copies of the ESR B allele appears to increase the fetal survival, total number of fetuses, total fetal weight, and number of fetuses per horn in the pregnant female. The overall effect of the B allele would therefore be an increase in reproductive performance, which has previously been demonstrated with both litter traits (Short *et al.*, 1997) and placental traits (Van Rens and van der Lende, 1998). The addition of more animals to this study should allow a final determination of the true validity and significance of these trends.

References

Haley, C. S., E. D'Agaro, and M. Ellis. 1992. Genetic components of growth and ultrasonic fat depth traits in Meishan and Large White pigs and their reciprocal crosses. *Animal Production.* 266:105.

Hughes, P. E. and M. A. Varley. 1980. *Reproduction in the Pig.* Butterworth & Co., Ltd., Boston.

Korach, K. S. 1994. Insights from the study of animals lacking functional estrogen receptor. *Science.* 266:1524.

Lehrer, S., M. Sanchez, H. K. Song, J. Dalton, F. Levine, P. Savoretti, S. N. Thung, and B. Schachter. 1990. Oestrogen receptor B-region polymorphism and spontaneous abortion in women with breast cancer. *Lancet.* 335:622.

O'Malley, B. 1990. The steroid receptor superfamily: more excitement predicted for the future. *Mol. Endocrinol.* 4:363.

Rothschild, M. F., R. Larson, C. Jacobson, and P. Pearson. 1991. *PvuII* polymorphisms at the porcine oestrogen receptor locus (ESR). *Anim. Genet.* 22:448.

Rothschild, M., C. Jacobson, D. Vaske, C. Tuggle, L. Wang, T. Short, G. Eckardt, S. Sasaki, A. Vincent, D. McLaren, O. Southwood, H. van der Steen, A. Mileham, and G. Plastow. 1996. The estrogen re-

ceptor locus is associated with a major gene influencing litter size in pigs. Proc. Natl. Acad. Sci. USA. 93:201.

Short, T. H., M. Rothschild, O. Southwood, D. McLaren, A. de Vries, H. van der Steen, G. Eckardt, C. Tuggle, J. Helm, D. Vaske, A. Mileham, and G. Plastow. 1997. Effect of the estrogen receptor locus on reproduction and production traits in four commercial pig lines. *J. Anim. Sci.* 75:3138.

SAS. 1990. *SAS/STAT User's Guide.* SAS Institute, Inc. Cary, N.C.

Van Rens, B. T. T. M. and T. van der Lende. 1997. The estrogen receptor gene and prolificacy in the pig. *Proceedings of the Wageningen Institute of Animal Science Seminar, "What Have We Learned from the Meishan Pig."*

Volatile Fatty Acid Emission as a Measurement of Perceived Odor from Swine Waste Compost Amended with Sawdust

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Abstract

Offensive odors evolved during the decomposition of swine waste have resulted in complaints from neighbors in agricultural areas. This study showed that the chronological monitoring of the formation of volatile fatty acids (VFAs) could be used in conjunction with the composting process to optimize conditions that minimize the release of malodorous compounds. Mixtures of swine waste and sawdust (3.5:1) were placed in 91 kg reactor vessels and constantly aerated over a 21-day period to chronologically monitor fermentation and formation of acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids. Composting conditions were shown to be adequate based on carbon to nitrogen ratios and moisture contents. The 21-day aerobic treatment decreased all VFAs retained in the biomass by 50 to 100% with an average of 87%. Chronological monitoring of volatile emissions from the vessels showed that prior to peak gas volatilization, when the vessels attained peak composting temperatures, acetic acid was emitted in the greatest amounts, followed by butyric, propionic, valeric, isovaleric, and isobutyric acids. Following peak gas volatilization, butyric acid accumulated in the greatest amounts followed by acetic and propionic acids, valeric, isovaleric, and isobutyric acids. Peak VFA emissions occurred simultaneously with the greatest headspace temperatures, peak rates of O_2 uptake, and peak production of condensate, ammonia, and CO_2. Therefore, stringent control of these factors may decrease VFA emissions. Fewer VFAs were emitted from those vessels that quickly heated and were active for short periods of time, whereas much greater amounts of VFAs were emitted over longer periods of time from those vessels that possessed adequate conditions for composting for longer periods of time.

Introduction

The major malodorous compounds in swine manure have been identified as volatile fatty acids (VFAs) including acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids, as well as the aromatic compounds phenol, *p*-cresol, indole, and skatole (Chen *et al.*, 1994; Schaefer, 1977; Williams, 1984). On average, slurries of swine waste have been shown to contain more VFAs than those of cattle waste (Cooper and Cornforth, 1978). However, laboratory experiments have indicated that

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VFAs persist only when oxygen is absent from waste. Rapid decomposition of VFAs occurred when air was passed through a waste sample, but decomposition occurred more slowly if air was passed only above the sample. When anaerobic conditions were re-established, VFAs did not reform unless protein hydrolysate or glucose was added, which suggested that aeration was effective to eliminate VFA precursors (Cooper and Cornforth, 1978). In addition, aeration has been shown to eliminate the production of methane and hydrogen sulfide and to reduce the production of ammonia (Stevens and Cornforth, 1974). The purpose of the present study was to determine whether the chronological monitoring of the formation of VFAs could be used in conjunction with the composting process to optimize conditions that minimize the release of malodorous compounds.

Materials and Methods

To chronologically monitor the formation of VFAs in conjunction with the composting process, identical reactor vessels were used. Approximately 200 lbs (91 kg) of composting mixtures of swine waste and sawdust (3.5:1) were placed in insulated 54 gal (200 L) stainless steel drums and continuously aerated for 21 days. Water vapor and volatilized gases in the headspace areas were condensed, and the liquid condensate was collected in separate polyethylene bottles over 12-hour intervals. In addition, VFAs within the vessel contents before and after the trial were extracted using slightly acidic nanopure water, centrifugation, and filtration. Quantitative analysis of liquid condensate samples and filtered extracts was performed using an HP5890 Gas Chromatograph (Hewlett Packard, Palo Alto, Calif.) equipped with a flame ionization detector.

In addition to VFA emissions, ammonia emissions, carbon dioxide generation, and oxygen taken up by microbial activity were monitored via the volatilized gases in the headspace areas of the vessels. Portions of the vessel contents were used to determine percent total carbon, percent total nitrogen, retained VFAs, and moisture contents.

Results and Discussion

The objective of the study was to recover and quantify the malodorous compounds from composting waste as well as from volatile emissions. Adequate composting conditions were confirmed using the carbon-to-nitrogen ratio, moisture content, oxygen availability, and aeration to displace excess heat generated by microbial activity. Microbial activity was monitored with O_2 utilization and CO_2 generation.

The greatest headspace temperatures and peak rates of O_2 uptake, as well as peak production of condensate, VFAs, ammonia, and CO_2, occurred simultaneously. As heat produced from microbial action increased the temperature, gas volatility increased, and VFAs and ammonia were released with water vapor. Throughout the trial, the moisture content of the composting mixtures decreased by 24 to 45% as a total of 4.54 to 6.95 gal (17 to 26 L) of condensed gases were produced per vessel. The vessels that produced the fewest VFAs also attained the highest internal temperatures and produced the greatest amounts of condensate (Figure 1). Peak ammonia production and peak internal temperatures were reached quickly, and the moisture released was sufficient to produce inadequate composting conditions that ceased microbial production of VFAs. However, in the vessels that heated and produced ammonia more slowly and over a longer period of time, the peak vessel temperatures were lower, and more than twice as much of each VFA was emitted (Figure 2). Thus, composting mixtures of apparently identical materials did not react to a given set of conditions equally. The conditions of the specific mixture influenced the amount of VFAs and, therefore, the amounts of odor emitted during composting.

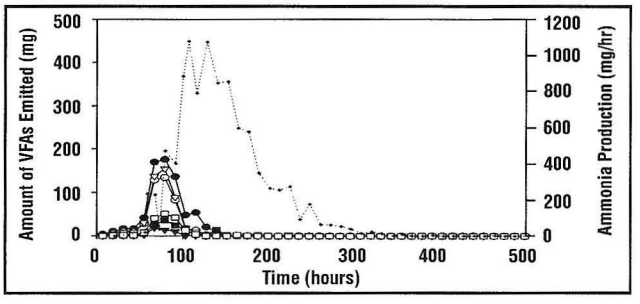

Figure 1. Average ammonia and VFAs emitted from those vessels that heated quickly and whose contents remained at elevated temperatures for a short period of time. (-●-) Acetic Acid, (-○-) Propionic Acid, (-▼-) Isobutyric Acid, (-▽-) Butyric Acid, (-■-) Isovaleric Acid, (-□-) Valeric Acid, and Ammonia (-+-).

In the compost vessels, acetic acid was emitted in the greatest amounts before peak production of condensate and peak composting temperatures, followed by butyric, propionic, valeric, isovaleric, and isobutyric acids, in order of decreasing emissions. After peak production of condensate, butyric acid was emitted in the greatest amounts followed by acetic and propionic acids which showed approximately equal emissions. Valeric, isovaleric, and isobutyric acids were produced in the least amounts throughout the entire trial. Extraction of solid samples obtained as the vessels were emptied confirmed that the 21-day aerobic treatment decreased all VFAs in the biomass between 50 to 100%, with an average of 87%. The reactor vessel apparatus was an effective method to measure malodorous compounds and ammonia emitted during the decomposition of livestock waste while simultaneously monitoring the environmental conditions of the composting process. In addition, aeration was confirmed to be an effective method for the control of VFAs and ammonia and, thus, odors produced by their emission.

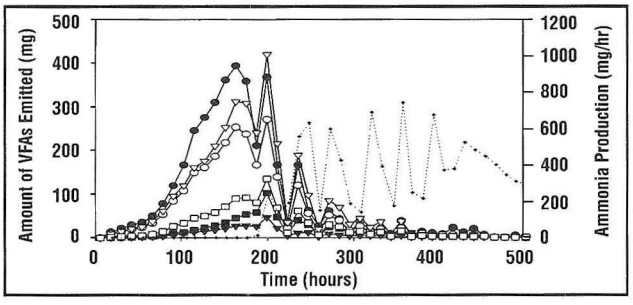

Figure 2. Average ammonia and VFAs emitted from those vessels that heated slowly and gradually and whose contents remained at elevated temperatures for a longer period of time. (-●-) Acetic Acid, (-○-) Propionic Acid, (-▼-) Isobutyric Acid, (-▽-) Butyric Acid, (-■-) Isovaleric Acid, (-□-) Valeric Acid, and Ammonia (-+-).

Conclusions

The results obtained in this study indicate that monitoring the chronological formation and fermentation of VFAs can be used in conjunction with the composting process to optimize conditions that minimize the release of malodorous compounds. Because peak emissions of VFAs occurred simultaneously with the greatest headspace temperatures, peak rates of O_2 uptake, and peak production of condensate, ammonia, and CO_2, stringent control of these factors may decrease VFA emissions. Fewer VFAs were emitted from those vessels that quickly heated and were active for short periods of time, whereas much greater amounts of VFAs were emitted over longer periods of time from those vessels that possessed adequate conditions for composting for longer periods of time.

References

Chen, A., P. H. Liao, K. V. Lo. 1994. Headspace analysis of malodorous compounds from swine wastewater under aerobic treatment. *Bioresour. Technol.* 49: 83-87.

Cooper, P., I. S. Cornforth. 1978. Volatile fatty acids in stored animal slurry. *J. Sci. Fd. Agric.* 29: 19-27.

Galliher, T. Testing Lab for the School of Natural Resources. The Ohio State University/Ohio Agricultural Research and Development Center, Wooster, Ohio. Personal Communication. March 1999.

Schaefer, J. 1977. Sampling, characterization and analysis of malodours. *Agric. Environ.* 3: 121-127.

Stevens, R. J., I. S. Cornforth. 1974. The effect of aeration on the gases produced by slurry during storage. *J. Sci. Fd. Agric.* 25: 1249-1261.

Williams, A. G. 1984. Indicators of piggery slurry odour offensiveness. *Agric. Wastes* 10: 15-36.

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